Handbook of
EQUINE PARASITE CONTROL

Craig R. Reinemeyer and Martin K. Nielsen

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Contents

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Preface

This book was conceived through the authors' realization that equine practitioners were not likely to achieve competence in evidence-based parasite control (EBPC) by reading journal-length articles or by attending a few hours of continuing education. Like any clinical skill set, parasite control must be grounded solidly in theory, practiced with thoughtful application, and continuously assessed and improved. Most new clinical skills, such as surgical procedures or diagnostic algorithms, represent - variations of basic proficiencies or knowledge already held by practitioners, who can also turn to local mentors for advice and support. In contrast, the private sector harbors few, if any, experts in equine parasitology who can impart mastery of the principles of EBPC.

Evidence-based parasite control is a relatively new development in equine medicine, but similar principles have been applied for decades by small ruminant practitioners in Europe and the southern hemisphere. In these locales, parasitic challenges to indigenous livestock are prevalent and extreme. Near-total anthelmintic resistance by certain parasites (e.g., *Haemonchus contortus*) has rendered practical control of these highly pathogenic nematodes nearly impossible, with severe economic consequences for the sheep and goat industries on multiple continents. In comparison, equine cyathostomins (small strongyles) have demonstrated resistance to one or more anthelmintic classes for nearly four decades, but these nematodes are modest pathogens under most circumstances. The authors and other veterinary parasitologists have been disseminating EBPC recommendations for many years, but equine practitioners have been relatively unreceptive to these messages until very recently. The impetus for this changed attitude is uncertain, but it may be associated with the contemporary detection of anthelmintic resistance in some populations of *Parascaris equorum*. The major threat perceived by practitioners is not likely to be mere demonstration of resistance in a second group of equine parasites. Rather, it could be the hard evidence that macrocyclic lactone anthelmintics, previously considered bulletproof panaceas in horses, are also vulnerable to nematode resistance.

Regardless of the motivation, equine practitioners now seem uniquely receptive to EBPC, and this book represents our attempt to address that interest and to fill the need with practical advice and logical recommendations. Most veterinary textbooks organize and discuss related facts, and then present recommendations for the logical application of that knowledge in clinical situations. This handbook has an additional objective that is far more daunting. The authors face the challenge of changing a mindset, of overcoming four decades of tradition, literally tens of millions of episodes of implementation, and competing recommendations from the marketing departments of every pharmaceutical company with a horse in the race. Change is painful but necessary, and progress in parasite control will be measured one practitioner and one horse owner at a time. As Darwin famously observed, "It is not the strongest of the species that survives, nor the most intelligent that survives. It is the one that is the most adaptable to change." The worms have been changing since the dawn of effective anthelmintic therapy; now it is our turn.

An exhaustive collection of references has not been included in this book because busy practitioners have neither the time nor the means to delve deeper into relevant literature. In addition, we readily acknowledge the irony that many of our "evidence-based" recommendations have minimal scientific support at present. So until more definitive proof is published, some practices clearly represent "stop digging" advice. (This term is derived from the adage that when you find yourself in a hole, the first thing to do is stop digging.) It is often a greater management challenge to convince people to stop doing the wrong thing than it is for them to adopt correct measures.

Our primary goal is to teach, and we believe that training in EBPC is best done by vet-side mentors. Accordingly, we beg the reader's indulgence whenever our tone becomes informal or even casual. This merely reflects our teaching styles.

Martin K. Nielsen
Craig R. Reinemeyer
October, 2012

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Section I

Internal Parasites and Factors Affecting Their Transmission

1 Biology and Life Cycles of Equine Parasites

Life cycles comprise the set of rules that parasites must obey as they interact with the environment and eventual hosts. A thorough knowledge of life cycles is not emphasized merely to torment veterinary students. Rather, life cycle details reveal opportunities to control parasites through chemical or management interventions, to exploit unfavorable environmental conditions, or to promote natural enemies that could be agents of biological control. Practical application of these approaches will be emphasized in individual chapters of this book.

At the root of all life cycles is a fundamental principle which distinguishes helminth parasites from other infectious agents such as viruses, bacteria, fungi, and protozoa. Through various types of clonal expansion, the latter can amplify their numbers within a host animal. Literally millions of individual organisms may arise from infective burdens that are orders of magnitude smaller. The reproductive products of helminths, however, are generally required to leave the host and undergo essential change in a different location. For most helminths, defecation is the most common means of exit, but exceptions include reproductive products that are ingested by bloodsucking arthropods (e.g., *Onchocerca*, *Setaria*). Some parasitic products become infective in the environment, others require intermediate hosts or vectors, but all, regardless, occur "outside the definitive host." Dramatic biological change is mandatory before the parasitic organism is capable of infecting a new host animal, or of reinfecting the original host.

Certainly more so than those organisms which amplify their numbers through clonal expansion, helminth disease is a numbers game. As the number of invading organisms increases, greater tissue damage or nutrient loss results, and the range and severity of clinical signs become more extensive.

In this chapter, we propose to describe the basic life cycles of the major helminth parasites of equids. Mention may be made of control opportunities revealed therein, but these will be discussed more fully elsewhere in the book.

Nematodes

Superfamily Strongyloidea

The members of the Strongyloidea ("strongyles") are moderately sized, stout worms with substantial buccal capsules. The males have a copulatory bursa at the posterior end, and females of all species produce eggs that are similar in appearance. Eggs of small strongyles cannot be differentiated microscopically from those of large strongyles, and the only practical method of differentiation (other than molecular approaches) is through coproculture. The strongyloids of horses all have direct life cycles; intermediate or paratenic hosts are never used (Figure 1.1).

Strongyloid eggs pass in feces and hatch in favorable environmental conditions of moisture, temperature, and oxygenation. All species exhibit three sequential larval stages, first (L_1), second (L_2), and third (L_3). The L_1 and L_2 stages feed on organic material in the environment, but the third stage develops within the sheath of the L_2. This protective covering helps L_3s to resistant environmental conditions, but it has no oral opening, and third-stage larvae are unable to ingest nutrients. The L_3 is the infective stage for all strongyloid nematodes of equids. Infection invariably occurs through inadvertent ingestion, whether while grazing or via oral contact with the environment.

It appears that horses never develop complete immunity to strongyloids, and they are often the sole nematode parasites recovered from well-managed, mature equids. The Strongyloidea of horses comprise two distinct subfamilies: the Strongylinae and the Cyathostominae.

Strongylinae (large strongyles)

Members of the subclass Strongylinae tend to be larger, on average, than most genera which comprise the Cyathostominae. In addition, Strongylinae have large buccal capsules, adapted for attachment to, and even ingestion of, the intestinal mucosa. Strongyline larval stages often undergo extensive, albeit stereotypic, migration within the host prior to returning to the gut to mature and begin reproduction.

Strongylus vulgaris

Strongylus vulgaris is widely acknowledged as the single most pathogenic nematode parasite of horses. The adult worms measure about 1.5–2.5 cm in length, and the females are larger than the males. Adults are usually found attached to the mucosa of the cecum and the ventral colon (Figure 1.2). After ingestion from the environment, third-stage larvae invade the mucosa of the distal small intestine, cecum, and colon. Here, they molt to the fourth stage (L_4), before penetrating local arterioles and migrating proximally beneath the intimal layer. Migrating *S. vulgaris* L_4s leave subintimal tracts in their wake and congregate near the root of the cranial mesenteric artery. A portion of the infecting larvae may continue to migrate, even to the root of the aorta near the left ventricle. Migrating L_4s have been found in numerous vessels arising from the aorta, including the renal arteries, and external and internal iliac arteries. The pathologic characteristics and consequences of these arterial lesions will be discussed in Chapter 2.

Figure 1.1 Strongyle life cycle. The life cycle of strongyle parasites. Parasitic stages can be seen above the horse and preparasitic stages below it. Fertilized eggs are shed by adult females in the cecum, and excreted to the environment in the feces. Here, the eggs hatch and a first-stage larva (L_1) emerges. The L1 then molts to L2 in the feces. Another molt gives rise to the L3, which retains its L2 cuticle and thus has a double-layered sheath. The L3 leaves the fecal pat and migrates onto forage, where it is ingested by a horse. Inside the horse, the L3 exsheathes and invades the mucosa of the large intestine. Large strongyles undergo extensive migration in various organs of the horse, while cyathostomins encyst in the mucosal lining of the large intestine. After returning to the large intestinal lumen, the worms reach sexual maturity and start shedding eggs

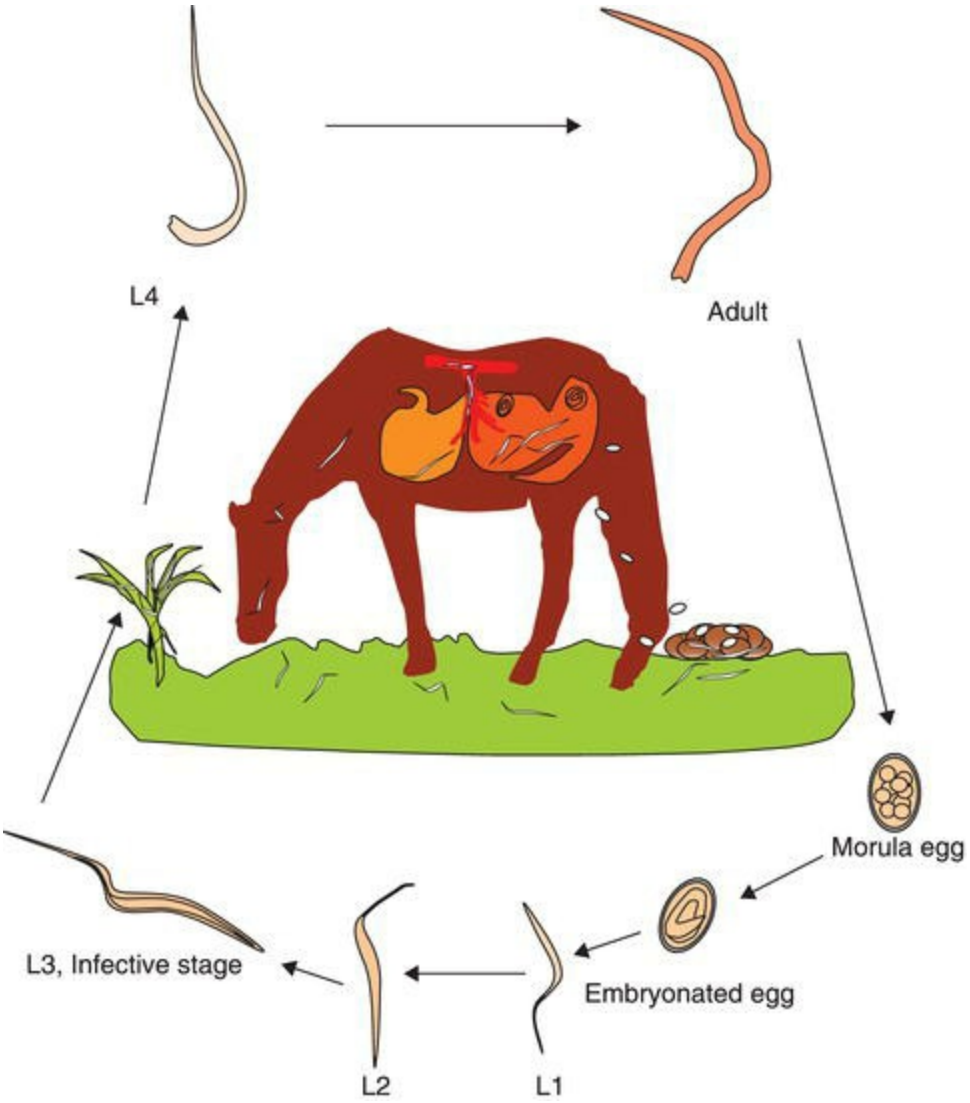
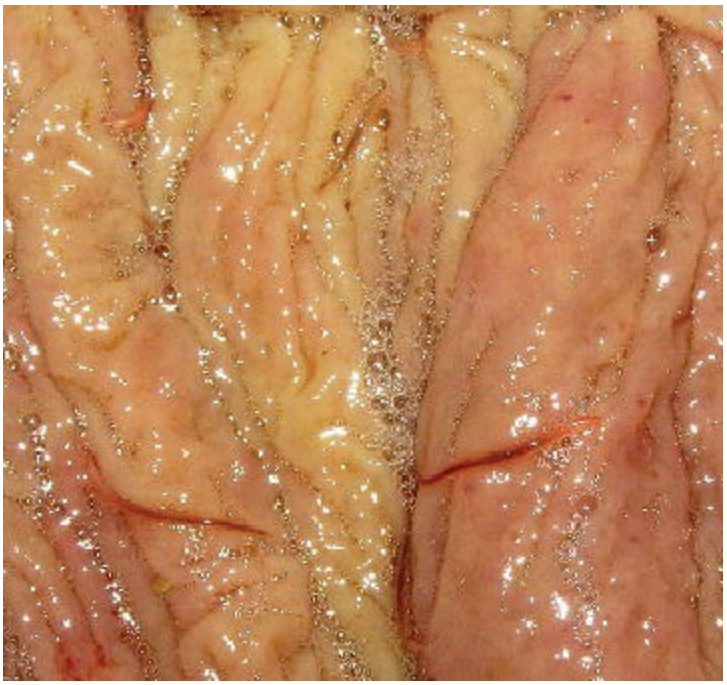


Figure 1.2 Adult Strongylus vulgaris attached to the cecal mucosa. (*Source*: Photograph courtesy of Tetiana Kuzmina)



Larvae reach the cranial mesenteric artery about 2 weeks postinfection. Here, they reside for about 4 months before returning to the large intestine. The final molt to the L_5 stage occurs about 90 days after infection, while larvae are still present in the artery. Young adults are transported by the bloodstream to the large intestine, where pea-sized nodules form around them in the submucosa of the ventral colon and cecum. Adult worms eventually emerge from these nodules and mature in the intestinal lumen for an additional 6 weeks. Females begin to lay eggs from 5.5 to 7 months after infection (Ogbourne & Duncan 1985).

Third-stage larvae of *S. vulgaris* are very distinctive in coprocultures and are easily differentiated from those of all other strongyloid nematodes occurring in horses (Figure 8.6). The diagnosis of *S. vulgaris* will be described further in Chapter 8.

Strongylus edentatus

Strongylus edentatus is a larger worm than *S. vulgaris*, measuring about 2.5–4.5 cm in length, and apparently is more prevalent. Adults are usually attached to the mucosa of the base of the cecum and the proximal ventral colon. The larvae undergo one of the most complex and fascinating migratory routes described in equine parasites. Following ingestion of infective L_3 stages from the environment, larvae migrate via the portal system to the liver, where they molt to the fourth stage within the parenchyma. Following migration within the liver, larvae migrate beneath the peritoneum via the hepatorenal ligament to various locations in the flanks and ventral abdominal wall (hence, the common term, "flank worm"). At necropsy, the large L_4s are found beneath the peritoneum covering various abdominal organs, and can also be located in isolated, hemorrhagic, and edematous lesions beneath the parietal peritoneum (see Chapter 2).

The final molt to the fifth stage occurs after about 4 months postinfection. Young adults migrate back to the large intestinal walls, where purulent nodules form and eventually rupture to release adult worms into the lumen. Altogether, this extensive migration results in a prepatent period of up to 1 year (McCraw & Slocombe 1978). The eggs of *S. edentatus* cannot be distinguished from those of other equine strongyles, large or small. Third-stage larvae of *S. edentatus* can be differentiated readily from those of *S. vulgaris* or the cyathostomins, but are fairly similar to the L_3 stage of the genus *Triodontophorus*.

Strongylus equinus

Strongylus equinus is another large strongyle with a prolonged life cycle, and a prepatent period of 8–9 months from infection to egg production. The adult worms are of about the same size as *S. edentatus*. The larvae molt to L_4 stage upon invading the mucosal layers of the cecum and colon. They are then described as migrating across the abdominal cavity and through the pancreas

to finally reach the liver, where they migrate for several weeks. On the way back to the large intestine, larvae again migrate through the pancreas and large L_4s and L_5s can be found free in the peritoneal cavity (McCraw & Slocombe 1984). The third-stage larvae are very distinctive in coproculture. This nematode species has become exceedingly rare in domestic herds. One author (CRR) has not recovered a single specimen of *S. equinus* from necropsies of hundreds of parasitized control horses over the past 25 years, and it typically is reported only from regions or equine populations with no or very sparse anthelmintic usage.

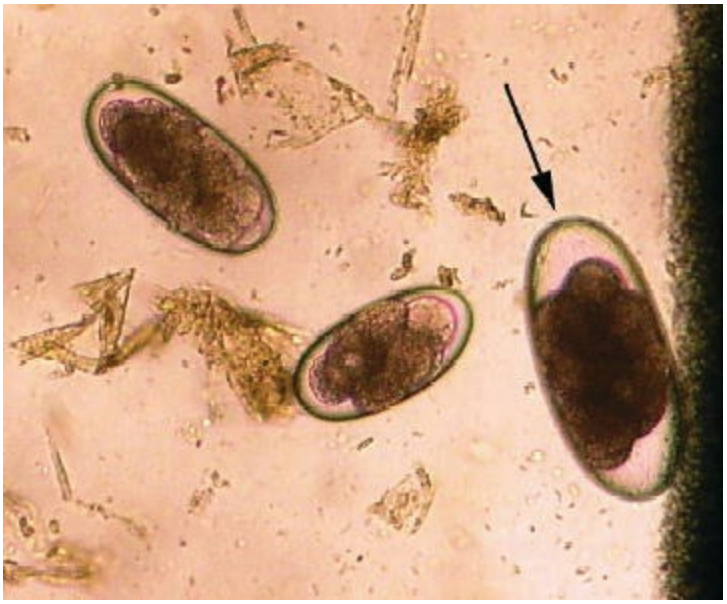
Strongylus asini

Strongylus asini is a common internal parasite of zebras and donkeys in Africa. It resembles *S. vulgaris* in many ways but genetically is more closely related to *S. edentatus* and *S. equinus* (Hung *et al.* 1996). Adults occur in the cecum and colon, but larvae are found attached to the lining of hepatic and portal veins (Malan *et al.* 1982). Fourth-stage larvae migrate within the liver and hepatic cysts are reportedly found in zebras. Peritonitis has been reported in association with migrating *S. asini* larvae (Jaskoski & Colglazier 1956).

Triodontophorus spp.

Although they are technically "large strongyles," the several species of *Triodontophorus* are nonmigratory. Their larvae encyst within the lining of the large intestine and emerge to become adults. The prepatent period is thought to be approximately 2–3 months (Round 1969). *Triodontophorus brevicauda* and *T. serratus* are probably the most prevalent species of large strongyles in managed horses, presumably because of a shorter life cycle than *Strongylus* species. Perhaps their privileged location in the mucosa protects them from the action of anthelmintics (e.g., ivermectin) that are effective against migrating strongylids.

Figure 1.3 Most strongyle eggs are very uniform in size. The only exceptions are the eggs of *Triodontophorus* spp. (arrow), which are about twice the size of a typical strongyle egg. (*Source*: Photograph courtesy of Tina Roust and Maria Rhod)



Triodontophorus females apparently produce eggs that are significantly larger than those of the other strongyline and cyathostomin genera (Figure 1.3). *Triodontophorus* larvae can be differentiated from other strongylid nematodes by coproculture (see Chapter 8). One species, *T. tenuicollis*, causes distinct pathology that will be described elsewhere.

Other Strongylinae

Craterostomum acuticaudatum, Oesophagodontus robustus, and Bidentostomum ivaschkini

These species have nonmigratory life cycles, and are only classified as Strongylinae on the basis of their large buccal capsules. The larvae derived by coproculture can be differentiated, but as the species prevalences are so low, larvae are more likely to be confused with similar, but more common, genera. None of these species has been associated with any distinct pathology.

Cyathostominae

The Cyathostomins (also known as small strongyles, cyathostomes, or trichonemes) comprise numerous genera, including *Cylicocyclus*, *Cyathostomum*, *Cylicostephanus*, *Coronocyclus*, *Cylicodontophorus*, *Gyalocephalus*, *Poteriostomum*, *Petrovinema*, and *Parapoteriostomum* in North America and worldwide. Lesser-known genera, such as *Hsiungia*, *Tridentoinfundibulum*, *Skrjabinodentus*, *Caballonema*, and *Cylindropharynx*, have been recovered from indigenous equids in Africa and Asia (Lichtenfels *et al*., 2008).

The basic life cycle of all cyathostomins is virtually identical, with development to the infective third stage in the environment. Once ingested by a horse, however, L_3 cyathostomins do not migrate systemically. (In this handbook, migration is used consistently to indicate leaving one organ and entering another.) Rather, incoming larvae invade the mucosa or submucosa of the cecum and ventral colon, or, to a lesser extent, the dorsal colon. Cyathostomins never encyst in the lining of the descending colon or rectum. Some species apparently invade no deeper than the mucosa, whereas others encyst within the submucosa. In addition, species may prefer certain alimentary organs or even sites within an organ for encystment.

Cyathostomins first invade the large intestinal lining as early third-stage larvae (EL_3). These are synonymous with infective larvae which have shed their protective integument. Early L_3s are small (< 1 mm) and most genera contain only eight intestinal epithelial cells. Soon after they enter the mucosa, a fibrous capsule, of host origin, forms around the EL_3, and from this stage forward, these tissue larvae are referred to as "encysted" (see Chapter 2). The EL_3 may be a transient stage, with immediate progression through other larval stages, or individual worms may persist as EL_3s for more than a year or two. The EL_3 is the one in which arrested development occurs.

With progressive development, the EL_3 molts into a late L_3 stage (LL_3), which is significantly larger, features a tubular buccal cavity, and has more than eight intestinal cells. The LL_3 remains within the cyst and ultimately molts into an L_4 stage, which has a distinct, goblet-shaped buccal capsule. The L_4 grows within the cyst, but ultimately the cyst wall ruptures and the L_4 enters the lumen of the large intestine. The stage of emergence is also termed "excystment," which is the chief pathologic event during the cyathostomin life cycle (Chapter 2).

The late L_4 (LL_4) within the large intestinal lumen grows in size and eventually molts into the adult, or L_5 stage. The adult develops within the sheath of the L_4 stage, and individual worms that are in the penultimate stage of development exhibit the buccal capsule and other cephalic features of the adult, positioned just inside the remnants of the L_4 stage, which are about to be shed and discarded.

In addition to the larval stages, adult cyathostomins also exhibit distinct site preferences (Table 1.1). Although it is not unusual for each organ of the large intestine to harbor at least some specimens of any species, the majority of individuals of any species are usually recovered either from the cecum, ventral colon, or dorsal colon. No species occupies the descending colon or rectum as a preferred niche, so specimens recovered from those locations are considered to be exiting the host.

Table 1.1 Examples of predilection sites of common cyathostomin species.

Source: Information from Tolliver (2000).

Cecum
Coronocyclus coronatus
Cyathostomum alveatum
Cylicocyclus elongatus
Cylicostephanus calicatus
Petrovinema poculatus

Ventral colon
Coronocyclus labiatus, *Cor. labratus*
Cyathostomum catinatum, *Cya. pateratum* (also dorsal colon), *Cya. tetracanthum*
Cylicocyclus auriculatus, *Cyc. brevicapsulatus*, *Cyc. radiatus*, *Cyc. leptostomum*, *Cyc. nassatus*, *Cyc. ashworthi*, *Cyc. ultrajectinus* (also dorsal colon)
Cylicodontophorus bicoronatus
Cylicostephanus asymetricus, *Cys. minutus*

Dorsal colon
Cyathostomum pateratum (also ventral colon)
Cylicocyclus insigne, *Cyc. ultrajectinus* (also ventral colon)
Cylicostephanus goldi, *Cys. longibursatus*
Parapoteriostomum euproctus, *Par. mettami*
Poteriostomum imparidentum, *Pot. ratzii*

Female cyathostomins can begin to lay eggs as soon as 5 weeks after infection (Round 1969), but due to arrested development, some may not complete maturation until more than 2 years after initial infection of the host (Gibson 1953). Cyathostomins can remain in arrested development longer than any other nematode species that spends its entire parasitic life cycle in the alimentary tract. The reasons for this strategy are unclear, but the evolutionary advantages are fascinating. If climatic conditions did not permit prolonged environmental survival, it would be very beneficial for the parasite if the host transported sources of new contamination and infection. Similarly, the same strategy would suffice if nomadic horses returned to the same grazing areas, but only after intervals longer than 1 year.

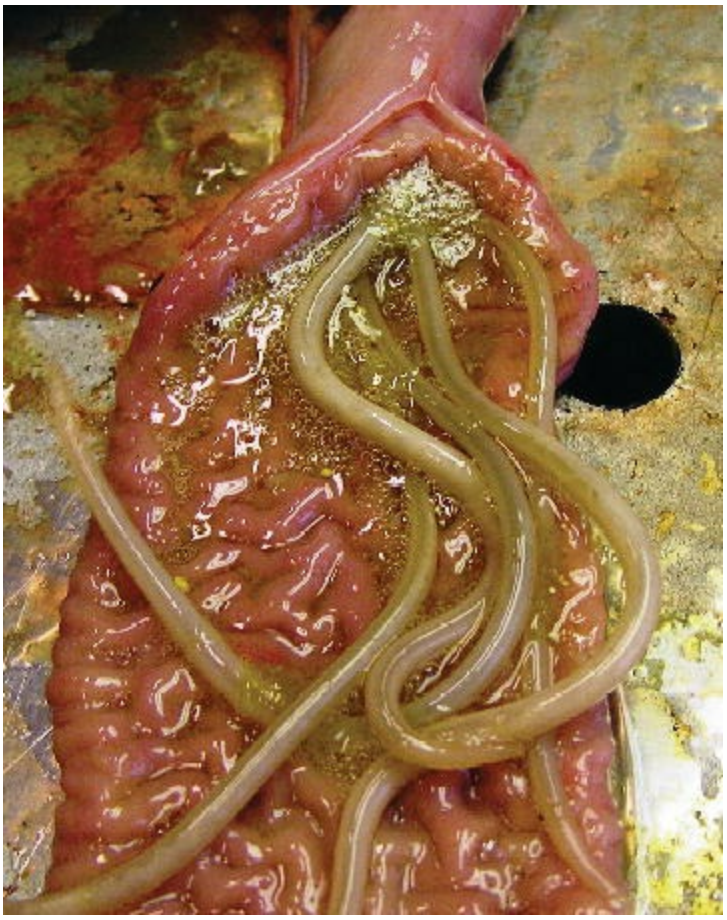
Encysted cyathostomin larvae are not 100% susceptible to any known anthelmintic regimen. For this reason, it is impossible to clear a horse of all its cyathostomins. If a horse were dewormed heroically and then transferred to a sterile environment with no hope of fecal/oral reinfection, that animal would eventually begin to pass strongyle eggs again at some point in the future. And, as demonstrated by Smith (1976a, 1976b), if the horse were held in such an environment for a prolonged period and dewormed repeatedly, it may require more than 2 years before the episodic contamination would cease permanently.

The duration of survival of individual cyathostomins has not been determined with certainty, but is thought to be on the order of 3–4 months.

Ascaridoidea

The superfamily Ascaridoidea comprises very large, stout nematodes with three prominent lips surrounding the oral opening. Ascarids have some of the most complicated life cycles of any nematode of veterinary importance, but the ascarid of horses has the simplest of all.

Figure 1.4 Adult *Parascaris equorum* in the small intestine of a weanling. (*Source*: Photograph courtesy of Tetiana Kuzmina)



Parascaris equorum

Parascaris equorum is the largest nematode parasite of horses, and mature females can reach 50 cm × 1–2 cm in size (Figure 1.4) and produce approximately 200 000 eggs per day. As adults, equine ascarids reside in the small intestine, with small numbers occasionally recovered from the stomach or cecum. Females lay distinctive eggs that are passed in the feces. Under favorable environmental conditions, eggs can become infective within 2 weeks. The infective stage is a larvated egg containing a coiled, second-stage larva. Once larvated, ascarid eggs can remain infective in the environment for up to 10 years.

Horses are infected by ingesting infective ascarid eggs from the environment. The eggs are covered by a sticky, protein coating which enables them to adhere to vertical surfaces, and even to the haircoat or udder of a mare. Foals and weanlings are most commonly infected by ascarids; and transmission is greatly assisted by the tendency of juvenile horses to investigate their environments orally.

When a larvated ascarid egg is ingested from the environment, the egg loses its protective coating after passing sequentially through the acidic and basic conditions in the stomach and small intestine, respectively. A larva emerges from the egg shell in the small intestine and penetrates the gut lining. Migrating larvae enter the lymphatics or venules draining the small intestine, and are carried passively to the liver. After infection, most larvae are found in the liver within 2–7 days postinfection. Larvae migrate within the hepatic parenchyma, which may result in inflammatory lesions and fibrous migratory tracts. Focal, white, fibrotic lesions are often seen just below the capsule of the liver (milk spots), similar to the condition caused in swine by migrating *Ascaris suum* (see Chapter 2).

Migrating third-stage larvae are found in the lungs beginning about 2 weeks after infection. Here, they exit the pulmonary venules and capillaries, and rupture alveolar membranes to enter the airways. Migrating ascarid larvae usually reside within the lungs for about 2 weeks. Eventually, the larvae migrate proximally in the pulmonary tree or are coughed up into the pharynx.

Regardless of the mechanisms, they are swallowed and return to the stomach and small intestine within 4 weeks postinfection. Once in the small intestine, the worms grow progressively, and eggs appear in the feces from 75 to 90 days postinfection.

Adult ascarids continue to grow and may persist within the gut for several months. Ultimately, the majority of horses develop very strong acquired immunity to *Parascaris*, and egg shedding eventually ceases, even without benefit of anthelmintic treatment. Because of this effective acquired immunity, ascarid infections are commonly observed in sucklings, weanlings, and yearlings, but are seen only occasionally in horses after approximately 18 months of age. Immunity is acquired, however, and not just attributable to the age of the host.

In recent years, many practitioners have observed patent ascarid infections in mature horses, and some individual adult horses resume egg shedding repeatedly after apparently effective anthelmintic treatments. At present, it is unknown whether this phenomenon occurs only in horses with unique immune deficiencies, or whether some isolates of *Parascaris* do not elicit a typical immune response.

Parascaris univalens

Few veterinarians are aware that a second species of ascarid exists in equids. *Parascaris univalens* differs from *P. equorum* primarily in that the genome of the former consists of only one chromosome pair, whereas the latter has two. This parasite was studied extensively in Europe during the late 1800 s, and it is an interesting item of trivia that the biologic phenomenon of mitosis was first observed in the eggs of *P. univalens*. More recently, the parasite has been used to study the phenomenon of chromatin diminution (Muller & Tobler 2000). Virtually nothing is known about the applied aspects of *P. univalens* parasitism. It is considered a cryptic (sibling) species, which cannot be distinguished morphologically from *P. equorum*, and some observations suggest that hybrids exist. It is assumed that many details of its life cycle are identical to *P. equorum*. However, it has not been established whether there are important differences between these parasites in their prevalence, pathogenicity, or the host's immune response to them. Also, it has not been determined whether these species differ in their ability to develop resistance to various anthelmintic classes.

Oxyuroidea

The Oxyuroidea, or pinworms, comprise a superfamily of nematodes which reside in the posterior alimentary tract. In addition to equids, other host species include humans, rodents, primates, and sheep. The oxyuroids have a unique biological adaptation in that the females do not shed eggs into the feces. Rather, they protrude from the anus and deposit eggs in a sticky film in the perineal area. The warm, moist conditions present there likely assist in larval development. Ultimately, the dried, proteinaceous film flakes off, and eggs are dropped randomly into the environment, where they may persist for several months.

Oxyuris equi

This is the common pinworm of horses. Adult females are white and moderate in size (5–8 cm × 5 mm, and have a sharply pointed tail—thus, the common name for this group). Males are fewer in number and only approximately one-third the size of the adult females. Adult pinworms reside in the descending colon and rectum, presumably so the females will have a shorter commute to the maternity ward.

Female pinworms may be seen protruding from the anus, but are also found in fresh feces or are observed adhering to a palpation sleeve following a rectal examination.

The larvated eggs are deposited in sheets of a sticky film, which is similar in composition to dried egg albumin. Eggs are ingested from the environment, in much the same fashion as those of *P. equorum*. Third-stage larvae emerge from eggs in the small intestine and reportedly develop within the mucosa of the cecum and colon. As pinworms approach adulthood, they relocate distally in the alimentary tract. Adults do not attach to the gut wall, and have negligible pathogenicity.

Diagnosis of pinworms is accomplished by demonstrating eggs in the perineal region, either by use of the scotch tape technique or by perianal scraping (Chapter 8). Pinworm infections are not likely to be diagnosed by routine fecal flotation because of their unique reproductive behavior. However, eggs are more likely to appear in a fecal sample that was collected directly from

the rectum. We attribute this to mechanical transfer of eggs to the surface of a lubricated glove (and thus onto the fecal samples) when the hand is inserted into the anus.

Probstmayria vivipara

Probstmayria is a lesser-known and extremely small pinworm that is occasionally recovered from horses. These worms are nearly invisible to the naked eye, but might be observed during microscopic examination of fresh colonic contents. This pinworm is not known to cause any distinct clinical signs. *Probstmayria's* reproductive behavior is rare among parasitic nematodes; it is viviparous and can complete its entire life cycle without leaving the host. For this reason, infections usually comprise massive numbers of worms, but again, they have no clinical impact.

Rhabditoidea

The rhabditoid nematodes are all fairly primitive, and exhibit unique life cycle adaptations such as free-living generations and an apparent absence of parasitic males.

Strongyloides westeri

Strongyloides westeri is a small nematode (6–9 mm) that parasitizes the small intestine of suckling foals. Females (parasitic males are unknown) are embedded within the mucosa at the base of the villi and produce small (50 µm × 40 µm), thin-shelled, round to slightly elliptical eggs already containing a larva. Patent infections are seen only in foals because absolute immunity is developed prior to around 5 months of age. Larvated eggs appearing in the feces of a yearling or older horse invariably are those of strongyles. Eggs pass in the feces, and L_1 larvae which emerge in the environment can follow various patterns of development. Some larvae become free-living males or females. The ones we are concerned about halt their free-living development as third-stage larvae, and are restricted to a parasitic existence.

Foals are infected with *Strongyloides* by one of three possible routes: skin penetration by third-stage larvae, ingestion of third-stage larvae from a contaminated environment, or lactogenic transmission from the mare. The latter route is possible because in immune adult horses, *Strongyloides* larvae do not become established in the alimentary tract. Rather, they are distributed to various somatic tissues, where they may reside for years. In mares, the hormones of pregnancy and lactation presumably stimulate the somatic larvae to resume migration and travel to the mammary glands. From this location, they are present in the mare's milk from the fourth day postpartum, and are ingested by her suckling foal (Lyons *et al.* 1973).

Most *S. westeri* infections in foals are asymptomatic, but symptomatic infections are described in Chapter 2.

Halicephalobus deletrix

Halicephalobus (syn: *Micronema*) is a free-living rhabditoid nematode that occasionally takes up residence within living tissues. It usually gains entry to the mammalian body through grossly contaminated lacerations or possibly through mucous membranes. *Halicephalobus* causes granulomatous lesions and is locally or systemically invasive. Spontaneous infections are seen occasionally in horses, and generally involve cephalic tissues (gingival and underlying bone, sinuses, brain) or well-vascularized organs such as the kidney (Ferguson *et al.* 2008). Human infections have been reported, but generally are subsequent to severe tissue damage and gross contamination with manure or soil.

Atypically for most parasitic nematodes, the adult worms reproduce within the host, resulting in superinfections with larvae in all stages of development. Anthelmintics are ineffective, and infections are invariably fatal.

Spiruroidea

All spiruroid nematodes require an arthropod intermediate host for transmission to a vertebrate vector. The spiruroids affecting horses either occur as adults in stereotypic locations, or as larvae in a variety of aberrant tissues.

Habronema muscae

Habronema muscae are approximately 1–2.5 cm in length, and occur in the stomach of equids. They produce very tiny (16 µm × 45 µm), thin-shelled, larvated eggs that are passed in the feces. In the environment, larvae emerge and are ingested by adult dipterans (e.g., *Musca domestica*), or are swallowed by feeding maggots. Infection is completed via ingestion of dead flies in feed stuffs or water. Alternatively, infective *Habronema* larvae may travel to the mouthparts of living flies, and be deposited in wounds or at mucocutaneous junctions during feeding activities.

Within the stomach, the parasites become adults in about 8 weeks. Adult *Habronema* are found in close contact with the gastric mucosa, but they cause no clinical problems. The larvae deposited in wounds or at mucocutaneous junctions, however, can result in proliferative lesions that grow and ulcerate throughout the fly season. This condition is known as cutaneous habronemiasis, or summer sores. The presence of spiruroid larvae can be verified through histopathologic examination of biopsied tissues.

Habronema microstoma

Habronema microstoma is a less common species within this superfamily that uses stable flies (*Stomoxys calcitrans*) as intermediate hosts. There are no major differences in the biology or pathogenicity of the two *Habronema* species.

Draschia megastoma

The life cycle of *Draschia megastoma* is virtually identical to that of the *Habronema* spp., and the house fly (*M. domestica*) is the preferred intermediate host. The major biological difference is that adult specimens of *Draschia* are found in large (5 cm × 5 cm), tumor-like, fibrous masses that are usually located near the *margo plicatus* of the stomach, which is the junction of the glandular and nonglandular gastric epithelium of equids. The historical prevalence of *Draschia* adults and associated lesions was 40% of 55 horses in 1984 (Reinemeyer *et al.* 1984). However, *D. megastoma* apparently has become quite rare because one of the authors (CRR) has not seen a single gastric lesion in hundreds of horses necropsied since 1985.

Thelazia lacrymalis

Horses are the definitive hosts of one species of *Thelazia*, or eye worms. As adults, *Thelazia* are found within the conjunctival *cul-de-sac* or beneath the nictitating membrane. Adult females produce larvae, which are present in the tear film of an infected eye. The usual intermediate host is the house fly, *M. domestica*, or face fly, *M. autumnalis*. Apparently, flies feeding on ocular discharges ingest larvae, which then develop to the infective stage within the body of the fly. Another horse is infected when the vector fly returns to feed on its lachrymal secretions. Infective stages leave the mouthparts of the fly, enter the conjunctival sac of the horse, and initiate a new infection.

Eye worms are thought to be relatively innocuous. They do not cause any direct damage, but might transmit pathogenic bacteria from one horse to another.

Filarioidea

The filarioidea comprise a superfamily of long, thin nematodes that often reside in organs with no direct connection to the external environment. So, these worms are challenged with distributing their reproductive products into the external environment so they can undergo the essential development necessary to infect a new generation of hosts. Filarioidea accomplish this goal by producing small, motile reproductive stages known as microfilariae. Microfilariae circulate in the blood or lymph, or migrate to the skin. From these locations, they are ingested by arthropod intermediate hosts which feed on the tissues or secretions of live horses.

Onchocerca

Onchocerca cervicalis and *O. gutturosa* adults are found deep in the connective tissues of the nuchal ligament, and those of *O. reticulata* occupy connective tissues in the distal limbs. Microfilariae are produced by female worms, and they enter the circulatory system and travel to the dermis and epidermis. Here, they are ingested inadvertently by *Culicoides* (midges) or *Simulium* (black flies) during their feeding activities. Microfilariae develop within the tissues of the fly, migrate to the dipteran mouthparts as infective L_3s, and reinfect another equid during subsequent feeding episodes. In the new host, infective stages migrate to the target connective tissues and begin reproducing approximately 6 months after inoculation. Infection can be diagnosed by demonstrating microfilariae in skin biopsy specimens incubated in saline.

The presence of adult *Onchocerca* is usually asymptomatic, but can be a nidus of persistent bacterial infections. The microfilarial stage in skin causes cutaneous onchocerciasis, which is characterized by localized itching, hair loss, and self-trauma, and may be exacerbated by treatment with effective drugs of the macrocyclic lactone class. Although macrocyclic lactone therapy kills microfilariae and temporarily sterilizes adults, mature worms eventually resume reproduction and the clinical signs of cutaneous onchocerciasis may ultimately recur (Sellon 2007).

Setaria equina

Setaria equina is a filarioid nematode that resides free within the abdominal cavity of equids. Although not pathogenic, it is a very prominent finding at necropsy which is hard to disavow in the presence of lay witnesses. Microfilariae are produced within the peritoneal cavity, but enter the circulation and can be found in peripheral blood. From here, they are ingested by feeding mosquitoes, and transmission is similar to that described earlier for the genus *Onchocerca*.

Parafilaria multipapillosa

Adult *Parafilaria* occur in subcutaneous and intermuscular connective tissue of horses. Nodules form in the overlying skin and may rupture and bleed or leak tissue fluids. First-stage larvae are present in the exudate from bleeding lesions, and are ingested by feeding horn flies (*Haematobia irritans*). Larvae develop to the infective third stage within the fly, and are transferred to horses when flies feed on lachrymal secretions or skin wounds. The larvae then migrate in the subcutaneous tissues and develop to the adult stage within a year. Eggs and microfilariae can readily be identified in smears taken from lesion exudates.

Trichostrongyloidea

Trichostrongyloids are fairly small nematodes which reside within the stomach or abomasum and small intestine of grazing animals. The free-living portions of the life cycle are virtually identical to those of the strongyloid nematodes discussed earlier. Most trichostrongyloids are parasites of ruminants.

Trichostrongylus axei

Trichostrongylus axei is the only nematode that horses share with other domestic animals. This parasite occurs in the abomasum of sheep, cattle, and goats, and there is some possibility of cross-infection among the various host species.

T. axei females reside in the stomach, and produce eggs which are deposited in feces. They are fairly similar to those of the strongyloid group, but tend to be slightly smaller, more delicate, and one end of the egg is somewhat pointed. *Trichostrongylus* infection can be diagnosed readily by differential coproculture (see Chapter 8). Horses are infected by accidental ingestion of larvae during grazing. Incoming larvae invade gastric glands and develop to the adult stage, whereupon they emerge into the lumen, and begin to lay eggs 3–4 weeks after infection. Certain horses develop massive infections of *T. axei*, involving thousands of individual worms. These horses exhibit hypertrophy of the glandular mucosa, but it is unknown if this condition results in any digestive disturbances.

In ruminants, *T. axei* infection is susceptible to anthelmintics of the benzimidazole and macrocyclic lactone classes; similar efficacy is likely in horses. However, a specific label claim does not exist for the equine products, due to the difficulty of demonstrating efficacy against infections of such low prevalence.

Dictyocaulus arnfieldi

Dictyocaulus arnfieldi is the lungworm of equids. Adults live in the terminal bronchioles and can be found in the major airways. Subsequent to reproduction, larvae deposited in the bronchial secretions are carried proximally to the pharynx by the ciliary apparatus or spontaneous coughing. The larvae are then swallowed and passed in the feces. Diagnosis involves using the Baermann technique to demonstrate larvae in the feces.

D. arnfieldi is considered a normal parasite of donkeys, because it reproduces readily and induces little pathogenicity. Horses, however, will rarely support an infection to the adult stage because they are not suitable definitive hosts. Thus, attempting to diagnose lungworm infection in a horse by demonstrating reproductive stages in the feces is a fruitless endeavor. Infection of a horse can be confirmed by a transtracheal wash to demonstrate eosinophilic bronchitis, or treatment with a macrocyclic lactone anthelmintic may be curative. Infected horses invariably have a history of sharing common pasture with donkeys.

Cestodes

Anoplocephalidae

Equids harbor only three species of cestodes, and only one of those can be considered common. All are members of a closely related family, and like nearly all other cestodes require an intermediate host for transmission. Unlike nematodes, equine cestodes apparently do not release individual eggs on a regular basis. Rather, terminal (gravid) proglottids probably detach and disintegrate during transit to the external environment. This results in a rather patchy distribution of cestode eggs within the fecal output of infected horses, with obvious diagnostic implications.

Figure 1.5 Anterior end of an *Anoplocephala perfoliata* specimen obtained from the cecum of a horse at necropsy. *A. perfoliata* is characterized by four suckers (arrows) with corresponding lappets (arrowheads) just beneath them. (*Source*: Photograph courtesy of Tina Roust and Maria Rhod)

In the environment, cestode eggs within feces are ingested by free-living soil mites of the family Oribatidae, which are endemic in soils worldwide. After ingestion, an oncosphere (essentially the scolex of a future adult worm) is digested from the egg within the alimentary tract of the mite. The oncosphere migrates into the hemocoel (body cavity) of the mite, and develops into an infective stage known as a cysticercoid. Cysticercoids probably remain infective for the life span of the mite host. It is likely that infected mites can persist in the environment for longer than a single climatic season.

Horses are infected via inadvertent ingestion of vector mites while grazing. The cysticercoids are digested free of the mite's tissue in the horse's gastrointestinal tract, and primitive scolexes attach to the lining of the preferred region of gut. Adult cestodes are able to regenerate an entire organism (known as a strobila) from the attached scolex.

Anoplocephala perfoliata

Anoplocephala perfoliata is the most common cestode of equids worldwide, and has been reported from every continent except Antarctica. It is a moderately sized worm, ranging from 4 to 8 cm in length and 1 to 2 cm in width (Figure 1.5). Unlike the cestodes of other mammalian species, it is rare to observe proglottids in the feces of horses, at least of those infected with *A. perfoliata*. *Anoplocephala* infection can be diagnosed by fecal examination, but this technique has fairly low sensitivity in horses, as discussed elsewhere (see Chapter 8).

A. perfoliata is a rare exception to the general rule that all adult cestodes reside within the small intestine of the respective host. Adult and developing *A. perfoliata* are mostly found attached to the lining of the cecum, and the majority tend to cluster on the cecal side of the ileocecal valve. It is not uncommon for additional masses of cestodes to be distributed in two or three locations within the cecum, and individual specimens can also be found attached to the mucosa of the ventral colon. The longevity of individual specimens of *A. perfoliata* is unknown, but is more likely to be months rather than weeks or years.

Several studies have demonstrated a clear, seasonal pattern in the prevalence and abundance of *A. perfoliata*. In temperate climates, most patent infections are observed in the second half of the year, reflecting infections that were acquired and established over the preceding grazing season (Meana *et al*. 2005).

Anoplocephala magna

True to its name, *Anoplocephala magna* is the largest cestode occurring in equids, and may achieve 80 cm in length. *A. magna* normally attaches to the mucosa in the distal small intestine, and can be differentiated from *A. perfoliata* by its relative size and preferred location in the host. For definitive identification of individual specimens, *A. perfoliata* exhibits small structures, termed "lappets," beneath each sucker, whereas *A. magna* lacks lappets (Figure 1.2).

Nearly a century ago, *A. magna* was reportedly far more prevalent than *A. perfoliata*, but the relative ranking of these species has reversed over time. At the present time, *A. magna* is encountered infrequently in North America.

Anoplocephaloides mamillana (formerly *Paranoplocephala mamillana*)

This is a very uncommon parasite of equids, which normally attaches to the mucosa of the proximal small intestine. It is a very tiny worm, only 6–50 mm long and 4–6 mm wide. The eggs differ in appearance and size from those of the *Anoplocephala* species. *Anoplocephaloides mamillana* is little more than a biological oddity and diagnostic differential; infections are not known to have any clinical impact.

Arthropods

Only one arthropod will be discussed herein, namely, members of the genus *Gasterophilus*, known commonly as bot flies.

Horse bot flies are members of a larger family, known as Oestrid flies. Although the details and the host distributions differ markedly, all oestrid flies employ the same general strategy, which is for their offspring to avoid unfavorable environmental conditions by passing their larval stages within the body of an intended host. The Oestrids of large domestic animals deposit eggs or larvae directly onto the intended host. Once the larval stage becomes active (after egg-hatching in some cases), they enter the host by specific routes. Thus, some Oestrids (e.g., *Hypoderma* of cattle) hatch from eggs attached to the haircoat, and the larvae penetrate intact skin and undergo sustained systemic migrations. Others (e.g., *Oestrus* of sheep) are deposited as larvae within the nares, and migrate only locally and develop within the sinuses. In most cases, the larvae overwinter within the host, emigrate from the host in spring, pupate in the soil, and emerge as adults to complete another generation. Most Oestrids are univoltine, meaning they propagate only a single generation per year.

Figure 1.6 ***Gasterophilus*** **spp. eggs attached to the haircoat of the leg.** (*Source*: Photograph courtesy of Tetiana Kuzmina)

Female flies of the genus *Gasterophilus* attach eggs to individual hairs of equid hosts (Figure 1.6), and larvae gain access to the oral cavity via routes that vary by species. Bot larvae generally overwinter within the equine alimentary tract, pass from the host in the feces during spring or early summer, and pupate in loose soil. Adult flies emerge from the soil 1–2 months later and emerge to mate and reproduce. Adult Oestrids have very brief life spans, due in part to their absence of mouthparts, which renders them incapable of ingesting nutrients.

Gasterophilus intestinalis

Gasterophilus intestinalis is the most prevalent and numerous of the bot species in domesticated horses. Female flies hover and glue individual eggs to hair shafts on the distal forelimbs, and occasionally along the neck and mane. Eggs hatch in response to contact with the horse's lips (Bello 1967), hatch immediately, and attach to the lips and tongue. Eggs which are laid on the mane are probably ingested by herd mates during mutual grooming. First-instar larvae are embedded within folds in the tongue. They subsequently molt to the second instar, and move to gingival pockets around the molars and premolars. The second instars eventually emerge, to be swallowed and passed into the stomach. Here, they attach to the mucosa in the nonglandular portion of the stomach and develop to the third instar. *G. intestinalis* larvae are dark red and spiny, and about 2 cm long and 5–8 cm wide. Burdens of several hundred bots are common in horses, and can be visualized easily and even enumerated gastroscopically. Features of bot pathogenicity are described in Chapter 2, and control is discussed in Chapter 7.

Gasterophilus nasalis

Female *Gasterophilus nasalis* flies deposit their eggs in the intermandibular area. The eggs hatch spontaneously and larvae crawl independently to the lips, enter the oral cavity, and develop in pockets in the tongue and around the cheek teeth. Ultimately, second instars are swallowed and develop further in the alimentary tract. Second and third instar *G. nasalis* prefer to attach in the ampulla of the duodenum, just a few centimeters past the pylorus. This area of the equine alimentary tract can be accessed and visualized endoscopically, so it is possible to detect this parasite antemortem.

Other Gasterophilus species

Other bot species which apparently do not occur in North America include *G. inermis* and *G. hemorrhoidalis*. The latter species attaches in masses in the distal small colon and rectum of donkeys in Africa, and has been documented as a cause of rectal prolapse. Other minor species are distributed around the globe, but none has distinctive pathogenicity.

Trematodes

Trematodes are uncommon parasites of horses in most developed countries. The liver fluke, *Fasciola hepatica*, occasionally infects horses, but is seen only in areas where fascioliasis is endemic in traditional, ruminant hosts. Horses with liver fluke infections inevitably have been pastured where microclimates favor the development of molluscan intermediate hosts. Readers are referred to Nansen *et al.* (1975) for a detailed description of the life cycle and clinical features of equine *Fasciola* infection.

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2 Pathology of Parasitism and Impact on Performance

Conventional wisdom maintains that parasitic organisms are inherently harmful, and the purported consequences of parasitism are legion (weight loss, diarrhea, anemia, hypoproteinemia, inflammation, etc.). However, there is a great distinction between parasitic infection (i.e., their mere presence) and disease. We all harbor *Escherichia coli* and *Staphylococcus aureus*, but these unbidden guests are tolerated as normal host flora until something goes out of balance. Somehow, the distinction between infection and disease seems much easier to ignore when the putative pathogen is grossly visible.

Unlike viral, bacterial, and fungal pathogens, nematode parasites (with rare exceptions) cannot amplify their numbers within the host. Consequently, the designation of parasitic disease amid the continuum of infection depends on the magnitude of exposure, and to the host's reaction (or inability to react) to the parasites and the changes they induce. Clinical parasitosis could be described as the culmination of thousands of micro-insults.

Animals vary widely in their susceptibility to parasitic disease, and certain individuals apparently experience far more damage from a standard number of parasites than a typical member of their species. In sheep and cattle parasitisms, it has been demonstrated that some elements of a host's ability to limit parasite numbers, and thus their susceptibility to disease, are determined genetically. But in addition, ancillary factors such as malnutrition, stress, immunosuppression, or concomitant illness famously predispose hosts to parasitic disease.

Parasitosis in an individual animal can develop from a variety of factors, some host and some management. When parasitic disease develops in a population of animals, however, the root cause invariably is related to management. The influence of various management practices on the size and potential damage of parasite populations is addressed in Chapter 6. The present chapter will focus on the pathogenic mechanisms specific to various helminth parasites of the horse, and the potential clinical manifestations in individual animals.

Nematodes

Strongylinae (large strongyles)

In the adult stage, large strongyles are found attached to the lining of the large intestine. Their large buccal capsules enable them to "inhale" plugs of mucosa, and to ingest nutrients in the form of blood, plasma, or mucosal cells. Although attached strongyles may cause focal inflammation and ulceration, the contribution of adult stages to strongyle disease is modest. Blood loss due to strongyle feeding is insufficient to cause clinical anemia because strongyles are rarely present in sufficient numbers to reduce the packed cell volume to dangerous levels. Rather, the major pathology caused by large strongyles can be attributed to their migrating stages (Ogbourne & Duncan 1985).

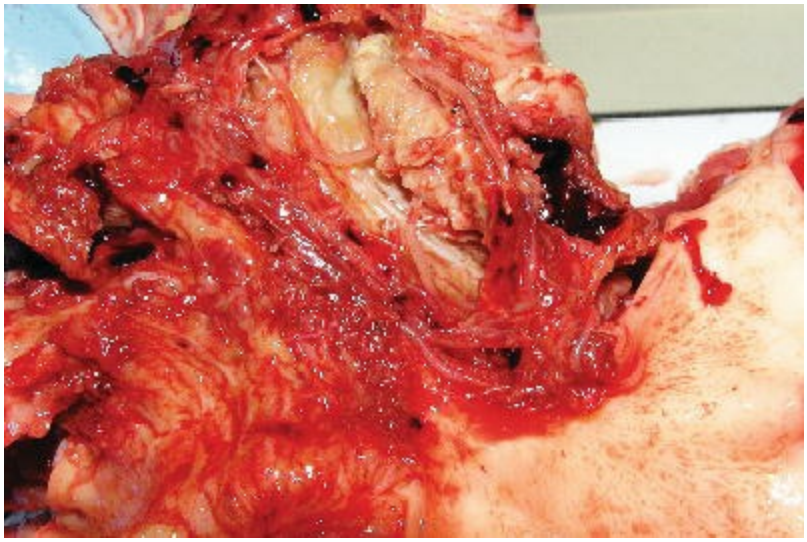
Strongylus vulgaris

The migratory pattern of *Strongylus vulgaris* was described previously in Chapter 1. The proximal migration of fourth-stage larvae (L_4) in the mesenteric arteries results in fibrous tracts beneath the intimal layer. After migrating larvae reach the cranial mesenteric artery and its major branches, they increase greatly in size and molt to the fifth stage. Their presence is associated with severe local arteritis, thrombi within the vessel lumen, and hypertrophy and fibrosis of the medial layer. The resulting enlargement of the root of the CMA can be palpated per rectum in small horses, and is a very prominent lesion at necropsy (Figure 2.1) (Ogbourne & Duncan 1985).

The incidence of colic has long been attributed to the prevalence of *S. vulgaris* and the associated arteritis, but formal evidence for this assumed correlation is anecdotal at best. It has been established that monospecific *S. vulgaris* infections in susceptible foals have resulted in severe and fatal disease. Despite the severity of the arterial lesions, the pathophysiology of the purported colic episodes is not clear-cut. A simplistic explanation is that thrombi arise from inflammatory granulation tissue, detach from the arteritis lesions, and are then embolized distally until they reach a terminal branch sufficiently small to become occluded (Enigk 1951). However, a postmortem survey of horses with ischemic bowel lesions failed to demonstrate emboli in the majority of cases (White 1985). It has also been hypothesized that *S. vulgaris* larvae cause colic by interfering with local neurologic control, which impacts gut motility (Wright 1972).

When fifth-stage larvae (L_5) are ready to return to the gut, the mesenteric circulation carries them distally to the large intestinal wall. The larvae exit the terminal arterioles and form fibrous abscesses within the wall of the cecum and ventral colon. These abscesses are approximately 5–8 mm in diameter, thick-walled, and filled with purulent material whether occupied by a larva or recently vacated.

Figure 2.1 ***Strongylus vulgaris*** **larvae at their predilection site in the root of the cranial mesenteric artery. Larvae are seen embedded in thrombus material, and the intima is roughened as a sign of endarteritis**



Strongylus edentatus

Following ingestion, *Strongylus edentatus* third-stage larvae (L_3) exsheathe in the small intestine, penetrate the gut, and migrate in the liver and retroperitoneal space. Larvae are commonly found free in the abdominal cavity or beneath the peritoneum along the body wall. The latter are grossly visible as 2–3 cm larvae embedded in discolored lesions, with evidence of hemorrhage and edema (Figure 2.2). Incising the peritoneum over these lesions typically releases a large, sluggish larva. Migratory *S. edentatus* larvae have been associated with liver pathology and peritonitis (McCraw & Slocombe 1978). The clinical impact of these migratory lesions is unknown, but they likely contribute to a general syndrome of strongylosis in heavily parasitized horses.

Macrocyclic lactone anthelmintics kill migrating *S. edentatus* larvae rather quickly, and within 1–2 weeks post-treatment, the retroperitoneal lesions contain dead larvae that are beginning to deteriorate.

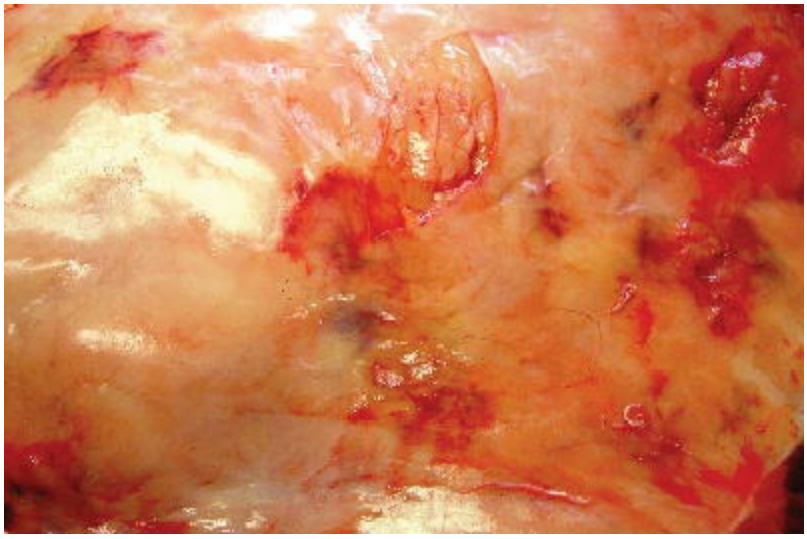
Mature larvae return to the colon by migrating beneath the peritoneum to the adventitial layer of the large intestine, and then migrating into the submucosa. Intramural abscesses develop, as for *S. vulgaris*.

Strongylus equinus

This large strongyle species has become extremely rare, so its inclusion here is merely for the sake of completeness. *S. equinus* larvae have a distinct preference for migrating within the pancreas and in the abdominal cavity before entering the liver. Mature

larvae eventually return to the gut in much the same fashion as *S. edentatus*. Pathological lesions include pancreatitis with subsequent pancreatic dysfunction, liver pathology, and peritonitis (McCraw & Slocombe 1984).

Figure 2.2 ***Strongylus edentatus*** **larvae in the ventral abdominal wall. Larvae migrate sub- and retroperitoneally and cause local hemorrhages. Incising these lesions reveals large strongyle larvae**



Triodontophorus spp.

Although *Triodontophorus* spp. are technically large strongyles (Strongylinae), their life cycle does not include a migratory stage, so they are more similar to the cyathostomins in that regard. Adults of the two most common species, *Triodontophorus serratus* and *T. brevicauda*, attach to the mucosa of the cecum and ventral colon, similar to the genus *Strongylus*. Although specimens of *Triodontophorus* are usually more numerous than *Strongylus* spp., they are substantially smaller and presumably cause little mechanical damage as adults.

One species, *T. tenuicollis*, causes pathognomonic lesions in the dorsal colon consisting of deep ulcers that are approximately 1–4 cm in diameter and tightly packed with very dark material. The ulcer contents peel away from the mucosa easily, but when the dark material is teased apart, one finds that it is comprised of tightly woven specimens of adult *T. tenuicollis*, often numbering several dozen in total (Drudge 1972). These "worm nests" are observed almost exclusively in horses less than 2 years of age. No specific clinical signs have been attributed to these verminous ulcers, and it is unknown whether test results for fecal albumin and hemoglobin would be similar to other causes of dorsal colonic ulceration. In any case, *T. tenuicollis* ulcers remain an interesting item of trivia for pathologists.

Cyathostominae (small strongyles; trichonemes)

Large strongyles were ubiquitous and virtually uncontrollable prior to the development of effective equine anthelmintics. In consequence, minimal if any pathogenicity was attributed to cyathostomins. However, as routine anthelmintic use eventually diminished the prevalence and importance of large strongyles, distinct syndromes of parasitic disease were recognized and ultimately attributed to small strongyles.

Cyathostomins are currently considered the most important nematode pathogens of mature horses. This distinction was not gained by overwhelming pathogenicity, but rather was earned largely by default: (1) large strongyles have been eradicated on well-managed farms; (2) other nematode pathogens (e.g., *Parascaris equorum*) are controlled by immunity and thus found almost exclusively in juveniles; and (3) the remaining, prevalent parasites of adult horses (e.g., bots and pinworms) are not very pathogenic. Although over 50 species of cyathostomins have been described, they are traditionally regarded as a homogeneous group with similar life cycles and common modes of pathogenicity.

Our current knowledge of cyathostomin pathogenicity is woefully inadequate, and the few details that are supported by scientific evidence are generally diluted by supposition and misinformation. The pathologic events and health consequences differ with the various stages of the cyathostomin life cycle, so these will be discussed chronologically.

Mucosal invasion

Infective third-stage cyathostomin larvae are ingested from the environment by grazing horses. Within the lumen of the small intestine, acid/base changes and enzymatic activity remove the protective sheath from each larva, revealing a minute (<1 mm) organism with only eight intestinal epithelial cells and a very small oral cavity. When the exsheathed L_3 reach the cecum or ventral colon, they enter the glands of Lieberkühn and penetrate cells at the base. These glands are deformed by the presence of growing larvae, and localized hypertrophy and hyperplasia of goblet cells is observed.

It is unknown whether the anatomic site of mucosal invasion is selected haphazardly by a particular species, or is influenced by a distinct organ preference. In a small sample of horses (Reinemeyer & Herd 1986), 57% of total encysted cyathostomins were recovered from the mucosa of the cecum, and 41% from the ventral colon. Less than 2% were found in the dorsal colon, which consistently is an organ of minimal involvement for larval cyathostomin development. Larval distribution has distinct pathologic relevance, as discussed later in this chapter.

Mucosal penetration by recently ingested L_3 has distinct pathologic consequences. It is logical that a macroorganism penetrating between or through mucosal epithelial cells would cause some mechanical damage. Invasion is accompanied by focal inflammation and a fibroelastic reaction in the lamina propria (Love *et al.* 1999), with perhaps an immune component in sensitized horses. Experimental infections have shown clinical pathology as soon as 3 weeks postinfection, suggesting that the mucosal invasion alone can be pathogenic (Love *et al.* 1999). The number and cumulative severity of these lesions should be greater in horses grazing heavily infective pastures, and would generally occur during seasons or climatic conditions which favor larval development or persistence, and likely both. In addition to climatic and seasonal factors, the risk of invasive damage would be compounded by factors which increase the numbers of larvae ingested, including overstocking of pastures and limited forage height.

The negative consequences of larval invasion can be minimized by limiting the numbers of infective larvae to which grazing horses are exposed. That topic is addressed in greater detail elsewhere in this book (see Chapter 6).

Encystment

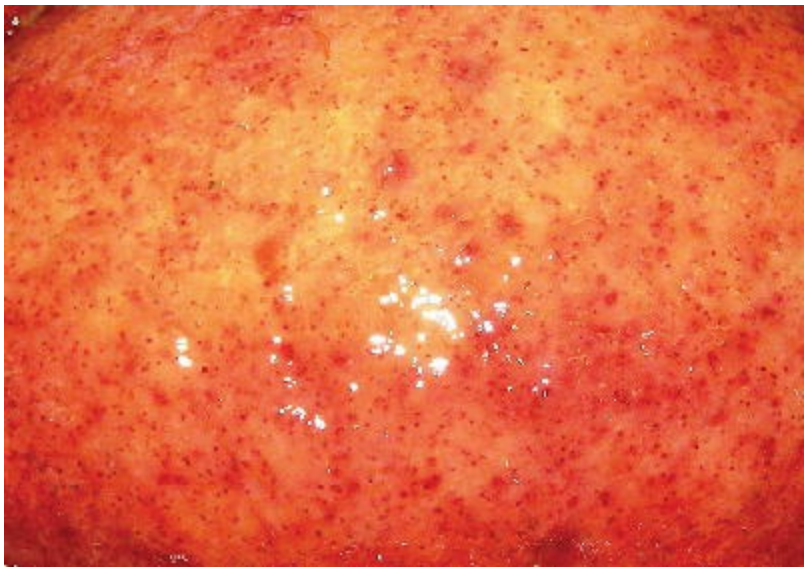
Following invasion of the mucosa, the L_3 of some cyathostomin species apparently penetrate no deeper than the mucosa, whereas other species invade the submucosa. In general, the latter tend to be larger cyathostomin species. Within days of mucosal penetration, a fibrous capsule of host origin develops around each invading larva, which can now be classified as "encysted" (Figure 2.3).

The cyst wall comprises the boundaries of each larva's universe for a period ranging from a few weeks to as long as 2.5 years (Gibson, 1953). Encysted larvae are constantly surrounded by a small volume of clear fluid, but the properties of this liquid are virtually unknown. The cyst wall apparently allows two-way passage of soluble materials. Because larvae increase greatly in size and maturity during their period of residence, it is obvious that nutrients of host origin must be able to penetrate the fibrous capsule. And, certain excretory products of the larva must be able to pass in the opposite direction because space within the cyst is insufficient to contain the volume that must be produced over 2 or more years. The host's protective mechanisms seem fairly oblivious to the presence of encysted larvae. Histopathology of encysted larvae reveals only modest inflammation around the cyst wall, as long as the structure remains intact (Love *et al.* 1999).

Another functional aspect is that the fibrous cyst capsule apparently prevents the entry of many types of anthelmintics. For example, pyrimidine salts and ivermectin have no apparent efficacy against encysted larval stages, regardless of dosage.

Moxidectin, however, demonstrates good but variable efficacy against encysted stages (Monahan *et al.* 1996), and benzimidazole anthelmintics (e.g., fenbendazole) are also effective when administered at an elevated dosage for several consecutive days (DiPietro *et al.* 1997). From a control standpoint, it is important to recognize that no anthelmintic regimen yet devised is capable of eradicating encysted populations. Consequently, horses can never be completely freed of their parasitic burdens, and they will always transport a future source of infectivity with them, even if confined in a pristine habitat (see Chapter 7).

Figure 2.3 Normal gross appearance of encysted cyathostomins within the cecal mucosa of a clinically healthy horse. Each dot represents one encysted larva. (*Source*: Photograph courtesy of Tetiana Kuzmina)



Some portion of the encysted larval population may reside within the gut tissues for 2 years or longer, as a consequence of a process known as arrested development. Arrested development is defined as a temporary cessation of parasitic development, at a specific stage in the life cycle, in response to certain environmental, host, or parasite factors. When arrest is terminated, the parasite resumes progressive development to the adult stage and, ultimately, sexual reproduction. Arrested development has been adopted by numerous nematode species, and is generally considered a survival strategy to avoid conditions that disfavor the immediate production of a successive generation. Cyathostomins arrest at the early third larval stage (EL_3) stage, apparently to avoid harsh climatic conditions that are inimical to the development and survival of infective stages in the environment. Accordingly, small strongyle populations in a northern temperate climate are likely to arrest through the winter months, emerging as fresh juveniles in late winter and spring. Conversely, cyathostomins in southern temperate climates tend to arrest through summer months, to avoid high temperatures. There is some evidence that cyathostomin populations in equatorial climates may not arrest at all (Eysker & Pandey 1987), presumably because local conditions are perennially favorable for translation.

Excystment

Patency can be accomplished in as little as 5–6 weeks when cyathostomin larvae undergo progressive development, that is, they develop directly and without interruption from an infective L_3 to the adult stage. The residence of larvae in the encysted stage is effectively limited to less than 1 month in this uninterrupted life cycle. Due to the effect of certain environmental or host conditions, however, larvae may remain encysted for over 2 years before they are ready to emerge into the gut and begin reproduction.

Emerging larvae presumably break through the cyst wall by means of a combination of mechanical and chemical factors, although none of the latter has been described. Emergence of larvae from mucosal cysts (i.e., "excystment") is the single most pathogenic episode of the cyathostomin life cycle. Late fourth-stage larvae (LL_4) are ten or more times larger than early L_3, so considerably greater mechanical damage to the gut lining ensues than when the larvae first invaded the mucosa as newly ingested

L_3. Substantial host reaction is mounted to the mechanical damage, but even greater is the putative host response to the excretory and secretory materials that were produced within the capsule during the period of larval development. Although the cyst contents have not been characterized, it is likely that they contain cytokines or other bioactive materials that elicit host inflammation. Love *et al.* (1999) describe eosinophilic infiltration around vacated cysts.

Sites of recent larval excystment exhibit hemorrhage, congestion, and edema. In horses with small cyathostome burdens, these lesions are focal and discrete. In horses with large populations, lesions are multiple and often coalescent. Horses with very large populations, especially during seasons when larval excystment is synchronous, may exhibit massive inflammation of the large intestine. The mucosa can become extremely edematous, 1–2 cm thick, and hemorrhagic. The clinical syndrome associated with these findings is termed larval cyathostominosis (Love *et al.* 1999; Peregrine *et al.* 2006). This syndrome is often characterized by profuse diarrhea, weight loss, anemia, hypoproteinemia, ventral edema, and dehydration. Larval cyathostominosis occurs in all ages, but is more prevalent in horses between 1 and 4 years of age and seems to coincide with winter/early spring reemergence of larvae in northern climates and summers in warmer climates. The case fatality rate is reported to be around 50% (Love *et al.* 1999), but larval cyathostominosis remains a rare event, considering that all horses have cyathostomin infections. Newly emerged cyathostomin larvae are occasionally observed in the feces, but this occurs in healthy horses as well. Thus, a definitive diagnosis is difficult to reach (see Chapter 8) because no single measure accurately characterizes the condition. Larval cyathostominosis can be compared to winter (Type II) ostertagiosis in cattle, and usually occurs in single animals within a herd. The overall management and deworming regimen used can be contributing factors to the occurrence of the condition, but given the uneven distribution of parasites within a herd, some horses will always be at more risk than others. The single most important risk factor is anthelmintic treatment within 1–2 weeks prior to the onset of disease. The immediate kill and removal of luminal parasites appears to trigger a synchronous emergence of encysted larval stages from the mucosal lining. The majority of excystment lesions are observed in the cecum and ventral colon, consistent with the known distribution of cyathostomin encystment. In typical infections, the mucosa of the dorsal colon remains relatively unaffected, and often serves as a reference tissue for "normal, uninfected" gut. Although numerous adult cyathostomins inhabit the dorsal colon, their presence does not appear to change the appearance or inflammatory status of the mucosa in that organ.

Table 2.1 Key to numerical scores for subjective assessment of inflammatory changes in the large intestine associated with encysted cyathostomins.

Score	Characterization
1	Mucosa uniformly thin and pale; no CHE
2	Mucosa generally thin and pale; focal lesions ofCHE adjacent to encysted nematodes
3	Mucosa displays diffuse, mild CHE, as indicated by darker coloration (red vs. yellow); focal CHE near encysted nematodes
4	Mucosa displays diffuse, moderate CHE, as indicated by thickening and brick-red color; CHE more intense near encysted nematodes, but also involves distant tissues
5	Mucosa displays diffuse, severe CHE, as indicated by marked thickening, intense hyperemia, and prominent edema; entire sample involved

CHE Congestion, hemorrhage, edema.

A numerical score was developed to characterize mucosal inflammation associated with cyathostomin populations (Reinemeyer 2003). The scoring system is a subjective measure of two cardinal signs of inflammation, that is, redness and swelling, and the pertinent pathologic terms would include congestion, hemorrhage, and edema (see Table 2.1).

Another parameter for documenting the pathologic impact of cyathostomin tissue stages is to express the gross weight of the cecum or ventral colon as a percentage of antemortem body weight. Theoretically, heavily infected large intestines exhibit greater inflammation, which is accompanied by more edema, and the presence of excess body fluids within the tissues results in a measurably higher organ weight.

Inflammation scores and organ weights were assessed in one study to compare changes in gut health status following treatment with larvicidal and nonlarvicidal macrocyclic lactone anthelmintics (Table 2.2).

Table 2.2 Comparison of mean mucosal inflammation scores and organ weights of control horses and those treated with ivermectin or moxidectin (*n* = 3/group).

Parameter	Organ	Control	Ivermectin	Moxidectin
Inflammation score*	Cecum	2.53	3.00	1.60
	Ventral colon	2.67	2.53	1.20
Organ weights†	Cecum	1.077	0.95	0.857
	Ventral colon	1.66	1.293	1.24

* See Table 2.1 for key.
† Expressed as a percentage of antemortem body weight.

In this small comparison study, untreated control horses had the highest average inflammation scores and the greatest mean organ weights. Numerical scores and mean organ weights were consistently lowest in horses that had been treated 5 weeks previously with moxidectin. The parameters of horses treated with ivermectin, which is not larvicidal, generally fell between those of the other two groups (see Table 2.2).

Adults

Adult cyathostomins are considered to be relatively innocuous even when present in extremely high numbers. Although some species may attach weakly to the mucosa, most reside in the paramucosal ingesta, where they feed on particulate organic materials. The gut contents of mature cyathostomes have been shown to contain ciliate protozoa, and even strongyle eggs.

Many cyathostomin species are known to exhibit distinct site/organ preferences as adults. For example, *Cylicostephanus longibursatus* adults occupy the dorsal colon almost exclusively, and often in very large numbers, whereas *Petrovinema poculatum* is rarely observed outside the cecum. Because so few larvae encyst in the dorsal colon, it is apparent that site/organ preferences are implemented only after the excystment and emergence of the adults.

Chronic cyathostominosis has been described as a clinical syndrome different than larval cyathostominosis. Whereas the latter is specifically associated with mass emergence of mucosal larvae, chronic cyathostominosis appears to be more generally related to the presence of various stages of the parasites within their hosts (Love *et al.* 1999). Symptoms are nonspecific, but include weight loss, dull haircoat, pot-bellied appearance, colic, and loose feces. Hypoproteinemia may be present, but is not a consistent finding.

Parascaris equorum

Because the life cycle of *Parascaris* involves migration within the host, this nematode has the potential to damage more than one organ system. After ingestion of larvated eggs, Larvae emerge in the lumen of the small intestine. They then migrate through the wall of the small intestine, enter local lymphatics, and are carried to the liver. Larvae migrate within the liver for approximately 1 week, resulting in inflammatory tracts, and white, fibrous scars ("milk spots") under the liver capsule, similar to those caused by *Ascaris suum* in swine. L_3 leave the liver approximately 1 week after infection, and are carried to the pulmonary circulation via the posterior vena cava. Larvae are trapped in terminal pulmonary arterioles or capillaries, rupture the vessel wall and enter alveoli, causing focal eosinophilic inflammation with edema and hemorrhage. Larvae migrate proximally within the airways or are coughed up into the pharynx. Migrating larvae generally leave the lungs by 2–3 weeks postinfection. Ascarid larvae are swallowed, and return to the gut, where they develop progressively from L_4 to adults within the small intestine. Adult ascarids may survive for several months, and continue to increase in size after achieving sexual maturity.

Migration of larval ascarids through pulmonary tissues (2–4 weeks after infection) may be accompanied by frequent coughing, and a grayish-white, purulent nasal discharge (Clayton & Duncan 1978; Srihakim & Swerczek 1978). The presence of ascarids in the alimentary tract may be accompanied by decreased feed intake, diarrhea, poor growth, rough haircoat, and weight loss or poor

weight gain. Ascarids do not attach to the mucosa, but apparently compete with the host for digested nutrients. Historical work with radio-isotopes demonstrated that parasitized foals had greater total body water and lower total body solids than control animals (Clayton *et al.* 1980). Infected foals also have lower serum concentrations and body pools of albumin.

Ascarids occasionally cause small intestinal obstructions, especially as a consequence of recent anthelmintic treatment. This is the sole example of mechanical bowel obstruction by parasites in horses. Small intestinal perforation is a known sequel, so ascarid impactions that cannot be relieved promptly by medical procedures will require surgery. Surgery to relieve ascarid impaction has a guarded prognosis, especially if postoperative ileus proves refractory to medical efforts to restore gut motility.

Practitioners should always be concerned about the possibility of ascarid impaction whenever history, clinical signs, or diagnostic results suggest that an individual juvenile might be harboring a large ascarid burden (see Chapter 7). No adverse events have been reported as a result of killing ascarid larvae as they migrate through the liver and lungs.

Oxyuris equi

Following ingestion of larvated eggs, L_3 *Oxyuris* invade the mucosal crypts of the cecum and ventral colon, where they develop to the L_4. Fourth stage larvae then emerge and feed on the mucosa until they reach the adult stage. Local mucosal inflammation has been reported to accompany larval invasion, but the clinical consequences of these observations appear to be minimal. Indeed, it would be difficult to discriminate such lesions from the more prevalent and numerous cyathostomin-induced lesions in the same tissues.

Adult pinworms are generally found in the distal large intestine and rectum. They do not attach to the mucosa, and their only routine pathology is secondary irritation from oviposition. Females discharge eggs onto the perianal skin of the host in a proteinaceous fluid. As this fluid dries, it apparently becomes irritating to the host. Consequently, horses rub their tail heads and rumps against fixed objects, causing local damage to the skin, haircoat, and tail. Horses rub their tails for numerous other reasons, so this behavior is not pathognomonic for pinworm infection.

Tapeworms

In large numbers, *Anoplocephala perfoliata* can cause severe inflammation at attachment sites around the ileocecal junction (Figure 2.4). Ulceration of the mucosa with formation of pseudomembranes has been reported, along with fibrosis of the muscularis layers of the cecum, and even transmural inflammation of the adventitial layer. Abattoir surveys have related tapeworm burdens to the degree of local pathological damage (Williamson *et al.* 1997; Kjær *et al.* 2007), but there is no corresponding evidence which correlates the degree of mucosal damage to clinical signs. Tapeworm burdens of 20 adult *A. perfoliata* or fewer generally cause little mucosal damage. Additional theories have been advanced that equine tapeworms hinder alimentary motility (peristalsis) by interfering with the local autonomic nervous supply of the gut (Wright 1972). Numerous case reports have associated tapeworm infection with ileocecal intussusception and even intestinal rupture (Barclay *et al.* 1982; Owen *et al.* 1989). A British epidemiological study reported significant associations between tapeworm ELISA antibody levels (see Chapter 8 for further description of this diagnostic method) and the risk of two defined types of colic: spasmodic colic and ileal impactions (Proudman *et al.* 1998). This study has been regarded as definitive proof of *A. perfoliata's* role as an equine pathogen. However, an attempt to reproduce the findings in Canada did not demonstrate any associations (Trotz-Williams *et al.* 2008), and further evaluations of the serum ELISA suggest a high level of background signal and many false-positive test results (Kjær *et al.* 2007). Thus, evidence indicates that *A. perfoliata* can cause disease in horses, but the health impacts of a typical tapeworm infection are still virtually unknown.

Figure 2.4 Adult *Anoplocephala perfoliata* at their predilection site around the ileocecal valve. (*Source*: Photograph courtesy of Tetiana Kuzmina)

Strongyloides

Strongyloides westeri infections are established during the first weeks of life, after larvae are transmitted to a foal via its mare's milk. Within the foal, larvae undergo pulmonary migration and return to the small intestine. Patent infections have been reported in foals as young as 5 days old (Dewes 1989).

Parasitic females are embedded within the mucosa of the small intestine, and can cause local inflammation. Cumulative gut irritation may be manifested clinically as diarrhea. Foals with clinical strongyloidosis display anorexia and lethargy, and a correlation between diarrhea and high egg counts (>2000 EPG) has been reported (Netherwood *et al*. 1996). Clinical signs are reversed by effective anthelmintic therapy, accomplished with ivermectin (200 µg/kg) or oxibendazole (15 mg/kg).

One differential to consider for any diagnosis of *Strongyloides* disease is foal heat diarrhea (FHD), which typically begins during the second week of life. Other than loose stools and "scalding" of the skin on the hindquarters, however, foals with FHD are fairly normal. They suckle well, and remain alert and active. Because *Strongyloides* infections remain asymptomatic, the observation of *S. westeri* eggs in a fecal sample from a young foal with diarrhea is by no means a clear-cut demonstration of cause and effect.

Specific anthelmintic treatment should be reserved for symptomatic cases of *Strongyloides* infection. Routine deworming of foals at 1–2 weeks of age to prevent or mitigate *Strongyloides* infections is discouraged. Such treatment is unnecessary from a health standpoint, and may help to select contemporaneously for anthelmintic resistance in *Parascaris*. Similarly, little benefit is gained by treating the mare with a macrocyclic lactone in the last month of gestation, with the intention of preventing lactogenic transmission to the foal. If foals do not acquire *S. westeri* infections directly from the dam, they are likely to be infected orally or percutaneously from the environment when the mare and foal are turned out to pasture.

A so-called "frenzy" syndrome has been described in foals and is associated with massive percutaneous penetration of *Strongyloides* L_3 (Dewes 1989). Affected foals had a sudden onset of stamping, walking quickly, circling and rolling in the mud, and scratching the face, ears, and neck with their hind feet. Episodes were 35 min or less in duration. Similar symptoms of distress were also noted in mares.

All foals develop absolute acquired immunity by the time of weaning, so strongyloidosis should not be considered as a differential for clinical diarrhea in weanlings or older juveniles.

Lungworms

Lungworm infection in horses is characterized by a chronic, productive cough, mucopurulent nasal discharge, and occasional fever. Clinical cases invariably feature a history of sharing pasture with one or more donkeys. The most pronounced pathologic finding is eosinophilic bronchitis, which, if chronic and severe, can result in a significant loss of lung function. As opposed to donkeys, horses are unsuitable definitive hosts for *Dictyocaulus arnfieldi*, so no or very few reproductive stages are produced to assist in diagnosis (see Chapter 8). Eosinophilia may be detected by transtracheal wash or alveolar lavage, but is not pathognomonic for lungworm infection. Infected horses generally respond well to therapy with macrocyclic lactone anthelmintics. A comprehensive management program would require treatment of resident donkeys and segregation of horse and donkey pastures in the future.

Trichostrongylus axei

Larval *Trichostrongylus axei* develop within gastric glands, and adults live in close contact with the mucosa of the glandular portion of the equine stomach. As in ruminants, the presence of large numbers of *T. axei* results in proliferation and hypertrophy of the gastric mucosa. Trichostrongylosis is not known to affect gastric pH in horses, and levels of plasma pepsinogen were not increased in infected horses in one study (Herd 1986). Although populations of *Trichostrongylus* can be maintained and perpetuated by horse herds, severe infections usually occur only in horses with a history of cograzing pastures with cattle or other ruminants.

Stomach worms (*Habronema* and *Draschia*)/spiruroid nematodes

Habronema spp. live in intimate contact with the gastric mucosa, but the adult stages are not known to cause clinical signs. In contrast, *Draschia* adults cause the formation of large, tumor-like nodules at the *margo plicatus*, but this parasite has virtually disappeared from North American horses in the past two decades. Otherwise, gastric infections have few manifestations, but cutaneous habronemiasis/draschiasis causes persistent, granulomatous lesions in skin wounds or at mucocutaneous junctions. Biopsy of suspected lesions of habronemiasis reveals eosinophilia, fibrous connective tissue, secondary bacterial infection, and ulceration. Cutaneous lesions can be treated with systemic macrocyclic lactone anthelmintics or excised surgically, but numerous alternative approaches have been employed in the past (Sellon 2007).

Eye worms

Thelazia lacrymalis is reported to infect conjunctival *cul-de-sacs* of horses on pasture (Lyons *et al.* 2006). Although infections are generally asymptomatic, cases of mild conjunctivitis and keratitis have been reported. One study reported abscess-forming dacryoadenitis in a stallion with chronic, recurrent bilateral conjunctivitis (Wollanke *et al.* 2004). Other infectious and allergic - etiologies can cause similar clinical signs, so multiple differential diagnoses exist.

Onchocerca

As adults, the three species of *Onchocerca* occurring in horses reside in the nuchal ligament or connective tissues of the distal limbs. Fibrous nodules occasionally form around the resident parasites in either location. *Onchocerca* spp. reproduce by forming

microfilariae that congregate in the skin, where they are ingested by the arthropod intermediate hosts of the genera *Culicoides* (midges) or *Simulium* (black flies). Microfilariae can cause chronic dermatitis or eye lesions that may be confused with allergic dermatitis (i.e., summer eczema) caused by hypersensitivity to *Culicoides* bites. Lesions of cutaneous onchocerciasis may persist perennially, or may increase in severity during seasons when the arthropod vectors are actively feeding. Systemic treatment with macrocyclic lactones is reported to have good efficacy against microfilariae, but up to 25% of treated horses experience pruritus or ventral edema in response to dead or dying microfilariae. Although not technically adulticidal, treatment with macrocyclic lactones apparently renders adult worms infertile for several months. Production of microfilariae inevitably resumes at some interval after M/L therapy, so repeated treatments may be necessary for long-term management of skin disease (Sellon 2007).

Setaria

Setaria are large, filarioid nematodes that are usually found free within the peritoneal cavity. These relatively large worms cause no apparent damage, although elevated levels of eosinophils can be found upon abdominocentesis and in blood samples. However, the latter may be associated with the microfilarial stage, which is present in the blood stream. Generally, *Setaria* parasites have no pathologic significance unless they wander into aberrant sites, such as the eye or central nervous system.

Arthropods

Gasterophilus spp.

First instar bots occupy fissures on the surface of the tongue, and second instar bots reside in gingival pockets at the base of molar teeth. Second and third instars of *G. intestinalis* are typically found attached to the mucosa of the nonglandular stomach (Figure 2.5), and those of *G. nasalis* are affixed to the mucosa of the duodenal ampulla, just distal to the pylorus. Attachment sites are characterized by large (1–2 mm) pits surrounded by hypertrophic mucosa. Lesions at attachment sites apparently have little negative impact on the host. Bot larvae do not cause gastrointestinal obstruction, nor do they contribute to gastric rupture. The greatest negative impact of botflies on their hosts might be the agitation horses experience during oviposition by females.

General impact of parasitism

Loss or diversion of nutrients

At the beginning of this chapter, we presented a partial list of abnormal clinical signs often attributed to parasitism. To that collection, we might add negative impacts on productivity and performance parameters, which vary widely for different types of horses. Performance for athletes differs from that of brood stock, and halter and conformation classes have different and unique requirements. In any case, these many and varied effects all require careful measurement and comparison; subjective assessments are not sufficient "proof" of parasitic damage. Many of the purported negative effects of parasitism on productivity are similar, if not identical, to those caused by nutritional problems. So, it is possible that parasitism exerts many of its general effects by interfering with the digestion, distribution, or utilization of nutrients, or that it limits the use of nutrients to maintaining homeostasis rather than contributing anabolic outcomes, such as increased bone and muscle mass, athletic fitness, etc.

Figure 2.5 ***Gasterophilus intestinalis*** **larvae attached to the nonglandular portion of the gastric mucosa.** (*Source*: Photograph courtesy of Tetiana Kuzmina)

This seems a logical hypothesis, but the only equine parasite for which interference with host utilization of nutrients has been documented is *Parascaris*. These very large worms physically compete with the host for the use of digested nutrients (amino acids, simple carbohydrates, and lipids) within the small intestine. Ascarids have been shown to ingest radiolabeled methionine when administered orally to infected foals (Clayton *et al.* 1980). The impact is more dramatic because ascarid prevalence coincides precisely with a rapid growth phase of the horse's lifespan. Foals harboring large numbers of roundworms simply cannot access the nutritional building blocks for optimal growth, metabolism, or performance.

Although nutritional deprivation or redirection are tempting hypotheses for other helminth infections of mature horses (cyathostomins, primarily), this is largely unsupported by facts and therefore much harder to explain. Adult cyathostomins apparently do not derive nutrients directly from the host mucosa, unlike large strongyles, and are relatively non-pathogenic compared to their earlier life-cycle stages within the host.

An issue that has not been investigated is the impact of encysted larvae on host nutrient cycling. Encysted cyathostomes increase in size tenfold or more during their development from EL_3 to late L_4, and the duration of residence of these resource burners may extend through many months if not years. Nematode growth requires critical nutrients, certainly amino acids and energy sources, and those are obviously derived from elements ingested by the host. Virtually nothing is known of the processes by which the cyst wall permits the influx of nutrients, and the excretion of waste products. Regardless of the mechanisms, most adult horses harbor literally thousands of encysted larvae, all of which utilize host nutrients on a constant basis. This implies that fewer critical nutrients will be available to support metabolic and homeostatic processes of the host.

Another potential source of nutrient loss is associated with the excystment of LL_4. The mucosal disruptions caused by excysting larvae initially hemorrhage, and presumably leak plasma thereafter until healed. This is a form of protein-losing enteropathy, and plasma protein spilling into the lumen of the large intestine probably cannot be recovered by the host. No digestive enzymes operate in the posterior gut to degrade insoluble proteins into simple amino acids that could feasibly cross the mucosa. Regardless of whether host protein is passed in the feces or degraded by local gut flora, it is no longer available to the host.

Finally, multifocal mucosal lesions initiate inflammatory cascades. As a consequence, antibodies and white blood cells are generated at the expense of the limited protein pools of the host.

Clinical health and productivity

Surprisingly little information has been published on changes in general health or production parameters in response to anthelmintic treatment or other effective forms of parasite control. One study investigated the incidence of colic in populations of horses kept under different anthelmintic treatment regimens in a long-term study, and concluded that macrolide lactones were associated with a lower incidence of colics (Uhlinger 1990). This suggests that strongyle infection constitutes a risk factor for colic in horses.

In another study, young, pastured horses that were treated with moxidectin or ivermectin achieved greater weight gains than an untreated control group (Reinemeyer & Clymer 2002), although the observed differences were not significant ($P < 0.05$) until day 120 post-treatment. A number of studies have evaluated body condition scores of horses and reported a positive association with anthelmintic treatment (Matthee *et al*. 2002; Crane *et al*. 2010).

Traditional notions that horses gain weight or improve body condition rapidly after effective deworming are unwarranted and simply inaccurate. (And, the converse reveals the fallacy behind subjective assessments that an anthelmintic treatment had obviously failed because the horse failed to "slick up" or gain weight soon after treatment.)

One study investigated strongyle fecal egg counts of Standardbred Trotters and evaluated the potential association with race performance. Surprisingly, the successful horses in the races had a tendency toward higher egg counts (Fog *et al*. 2011). Thus, the horses in this study appeared to be unaffected by the presence of moderate strongyle burdens. The effects of parasitism on other types of performance have not been documented objectively, nor have improvements thereof in response to anthelmintic treatment or other control measures been described in any detail. Yet, persistent presumptions support many traditional practices, including frequent deworming of race horses, use of daily dewormers in halter horses, etc. Objectively, there is very little proof one way or the other. These are classic examples of uncontrolled experiments: "I always do such and such, and I'm happy with the results, so it obviously works." And therein lies perhaps the greatest obstacle to changing prevalent attitudes about the importance and methods of parasite control.

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3 Environmental Factors Affecting Parasite Transmission

As mentioned in Chapter 1, a key feature of parasitism is that offspring must visit the environment to undergo essential changes before they are capable of infecting a new generation of hosts. Domestic horses have been imported to nearly every continent, where their parasites are exposed to differing environments with varied climatic conditions. Regardless, certain common rules apply to understanding parasite ecology, which is the relationship of parasites to their environment. This chapter summarizes the effects of environmental factors on egg hatching, larval development, and survival of infective stages. A thorough understanding of these principles will inform practitioners not only of *where* and *how* their patients become infected, but of equal importance, *when*.

Parasite *refugia*

Preinfective and infective stages in the environment typically represent more than 99% of the entire "parasite" population in grazing horses and their pasture habitat. Because the entire environmental assemblage was derived from eggs passed by adult worms in the host, both subpopulations share similar genotypic information. This is an extremely important concept to grasp in understanding the genesis (and prevention) of anthelmintic resistance.

Although environmental stages are not technically "parasitic" until they become established in a host, the following discussion will refer to the entire worm population on a farm as "parasites." *Refugia* is defined as any portion of a population that is not exposed to a selection pressure. The selection pressure of greatest importance in parasite control is anthelmintic treatment. Those 99% of the parasites resident in the environment are not exposed to anthelmintics whenever a host is dewormed, so that the entire population can be classified as "in *refugia*." Other examples of *refugia* include parasites in untreated individuals and certain parasitic stages, such as encysted cyathostomins, that do not come into contact with the drug. It is postulated that the single most important factor affecting development of anthelmintic resistance is the size of the parasite *refugia* (van Wyk 2001). By escaping selection pressure, the parasites in *refugia* comprise a valuable source of susceptible gene alleles to dilute resistant alleles in the population. Accordingly, maintenance of adequate parasite *refugia* can reduce the rate of development of anthelmintic resistance. This has been confirmed by experimental studies with sheep (Martin *et al.* 1981; Dobson *et al.* 2001; Waghorn *et al.* 2008) as well as by computer simulation modeling (Barnes *et al.* 1995).

Table 3.1 Survival of free-living equine strongyle stages when exposed to different climatic influences (Nielsen*et al.*, 2007).

Free-living stage	Frost	Alternation between frost and thaw	Desiccation	Heat[a]
Unembryonated egg	++	++	[b]	++
Embryonated egg	+	–	[b]	++
First stage larva	–	–	–	++
Second stage larva	–	–	–	++
Third stage larva	+++	+	+++	–

- indicates very susceptible, + weakly resistant, ++ moderately resistant, +++ very resistant
[a] Temperatures in the range of 30–38°C.
[b] No data available

Although it is difficult to accurately measure the size of *refugia*, the rate of development of anthelmintic resistance should be slower in larger, compared to smaller, *refugia*. Environmental conditions are the most critical factor in the development and persistence of infective stages, so climatic factors are the key determinants of the size of the *refugia*. Although soil type, vegetation, and stocking rates also play important roles, the two most important environmental factors are temperature and moisture. The different free-living stages of strongyle parasites are affected differently by these factors, as summarized in Table 3.1. The chronology of free-living stages is illustrated in Figure 1.1.

Preparasitic development

For purposes of the following discussion, a distinction will be made between preparasitic development and persistence. *Development* includes the processes of egg hatching and sequential progression through the first (L_1), second (L_2), and third (L_3) larval stages. *Persistence* refers to the duration of survival of L_3 stages in the environment.

Effects of temperature on development

Strongyle eggs generally hatch at temperatures above 6°C (43°F), and larval development occurs up to about 40°C (104°F). The optimum temperature for development of eggs and larvae is in the range 25–33°C (77–91°F), at which all developing larvae reach the infective L_3 stage within 3–4 days, with the highest larval yield at 28°C (82°F). No egg development is observed at temperatures below 4°C (39°F), while hatching takes 12–14 days at 6–10°C (43–50°F), 2–7 days at 10–20°C (50–70°F), 1–2 days at 20–30°C (70–86°F), and less than a day at 31–38°C (86–100°F) (Nielsen *et al.* 2007).

Effects of freezing on development

Myths and misconceptions are common regarding the survival of free-living strongyle stages in freezing temperatures. The expression "killing frost" has been applied to insects and plants, and it is often erroneously assumed that it describes what happens to preparasitic stages as well. Although freezing temperatures can have certain effects on eggs and larvae on pasture, the killing effect has been shown to be rather limited under practical circumstances (Nielsen *et al.* 2007).

Several laboratory studies have determined that long-term freezing damages strongyle eggs and reduces larval yield significantly. The various free-living stages appear to differ in their susceptibility to cold. Unembryonated eggs appear to withstand frost better than embryonated eggs, while L_1 and L_2 larvae were most susceptible to freezing. L_3 were generally the most resistant to cold. More than 90% of all L_1 and L_2 died after 1–4 days at −6°C to −10°C (21°F to −14°F) (Nielsen *et al.* 2007).

To understand conditions in the field, it is also relevant to consider the effect of alternation between frost and thaw, which often occurs during winter in temperate climates. Alternate freezing and thawing has a deleterious effect on most stages of strongyles but unembryonated eggs were relatively unaffected by up to 97 days of occasional freeze/thaw cycles. In comparison, embryonated eggs and L_1 and L_2 succumbed under identical conditions. Freeze/thaw cycles can be mitigated somewhat by the effects of snow cover. A few centimeters of snow on the ground shelters the strongylid microenvironment, and prevents wide variations in temperature. Under the snow, temperatures on the ground surface effectively remain close to 0°C (32°F). Thus, snow cover is protective for unembryonated eggs, and an intact fecal ball apparently provides additional security against fluctuating temperatures.

Effects of moisture on development

Moisture is another critical requirement for larval development of equine strongyle larvae. The lower limit for successful larval

development appears to be 15–20% moisture in the feces, and optimal fecal moisture content was identified as 57–63%. One study found that L_1 survived for only a few days in feces that were rapidly desiccated. In contrast, slower desiccation supported development to the L_2 stage, and a high percentage of these larvae survived and were capable of resuming development to L_3 when moisture was eventually supplemented (Nielsen *et al*. 2007).

Preparasitic persistence

Because persistence describes the duration of survival of L_3 in the environment, it also represents a measure of the risk of infection. The quantitative term for describing the numbers of L_3 on pasture is *infectivity*.

Energy reserves

In order to appreciate how L_3 survive the various environmental influences described previously, it is necessary to understand their energy metabolism and storage. L_3 are surrounded by a protective membrane, which is the discarded covering of the L_2. This membrane, or sheath, completely surrounds the L_3, effectively preventing it from ingesting any nutrients. As a consequence, L_3 must survive solely on energy reserves stored in their intestinal cells in the form of lipids and carbohydrates. If these larvae are highly active, they quickly use up the energy reserves and expire if not ingested by a horse. However, if larvae are less active, they can survive much longer because they do not burn up their limited energy reserves. Conditions which permit high larval activity include warm temperatures of 30–40°C (86–104°F) and high moisture levels or water films in which to swim. In contrast, cold weather and/or desiccation restrict the movement of larvae, so they can survive for very long periods. Interestingly, larvae seem to resist even very hot weather if they remain in a desiccated state which does not allow mobility. In studies performed in Texas and Australia, no larvae could be recovered from pasture herbage samples during dry periods, but subsequent rainfall allowed larvae to leave desiccated fecal balls and migrate onto the forage.

Effects of temperature on persistence

Myths and misconceptions are common regarding the survival of free-living strongyle stages in freezing temperatures. Several laboratory studies have determined that L_3 survived for longer intervals at 3°C (37°F) and −5°C (23°F) than at 31°C (88°F) and 26°C (79°F). Another study reported that freezing for 30 min or 72 h had no effect on L_3, whereas constant freezing for 5 or 8 months markedly reduced their survival (Nielsen *et al*. 2007).

A proportion of L_3 can tolerate alternating temperatures, but less than 1% survived five occasions of freezing for one to 5 days interrupted by thawing for a few hours. The previous comments about the protective effects of snow cover apply to L_3 as well, perhaps more so than to unembryonated eggs. When horses in northern temperate climates are turned out to spring pastures that have not been grazed since the prior autumn, strongyle larvae will be out there waiting for them. Some larvae will have survived the entire winter as L_3, whereas others have developed recently from unembryonated eggs fortunate enough to find a protective microenvironment.

Effects of moisture on persistence

Moisture is also a critical factor in determining persistence of strongylid larvae. Larvae prefer to migrate in water films, and can only be mobile if there is sufficient moisture present in their microenvironment. It has been reported that strongyle larvae survived markedly better in intact versus disrupted fecal balls, and L_3 kept on a glass slide survived desiccation in an incubator at 30°C for 65 days.

Apparently, under certain temperature conditions, desiccation can protect rather than kill L_3. Several reports document that L_3

in a desiccated state withstand freezing better than larvae that were kept moist. One feasible explanation for these observations is the quantity and size of ice crystals formed during freezing. Ice crystals are capable of disrupting eggs and larvae, so it is logical that the combination of freezing and dry conditions would afford some protection.

Role of fecal balls

Fecal matter provides a highly protective habitat for eggs and larvae. When fecal balls remain intact, some moisture can be retained under the hardened surface and larvae are protected from ultraviolet irradiation, and somewhat from temperature fluctuations. These effects have been documented under field conditions in cold climates. The protective nature of intact feces supports the recommendation to spread manure (by harrowing, mowing, or dragging pastures) at the end of the autumn grazing season if the pasture can be left unoccupied through winter. Long-term exposure of unprotected larvae to freezing temperatures or alternation between frost and thaw will most likely kill the majority before the following grazing season unless snow cover is nearly continuous.

Other parasites

Environmental conditions exert slightly different effects on the preparasitic stages of other equine parasites. A brief discussion of the more important parasites follows.

Parascaris equorum

Ascarids have a direct life cycle just like the strongyles, but achievement of infectivity does not require their eggs to hatch in the environment. Instead, horses are infected by ingesting embryonated eggs. Embryonated eggs persist quite well in deep litter, on surfaces in stalls, and on feeding equipment, and can be recovered from the perineum and udder of mares. Ascarid eggs are additionally very resistant to most chemical disinfectants, including formalin and strong acids. Ascarid eggs are purported to remain viable in the environment for up to 20 years, but this would be the exception rather than the rule. However, eggs of *P. equorum* can be expected to survive for 1–5 years in a typical stable/farm environment. Recent studies have shown that soil type is critical for egg survival. Gravel paddocks are much less favorable for persistence of ascarid eggs than soil types containing more organic matter (Ihler 1995). Studies with swine ascarid eggs (*Ascaris suum*) have demonstrated that plowing pastures may provide a temporary respite from ascarid infection. But, the benefit persists only until the pasture is plowed again in the future, when the eggs will be returned to the soil surface, just as viable as before (Mejer 2006).

Infection with *P. equorum* can be considered a “foal-to-foal” disease, in which foals born 1 year shed eggs into the environment, where the eggs persist until next year’s foal crop arrives and becomes infected.

Oxyuris equi

Little is known about the eggs of *Oxyuris equi*, the equine pinworm. Physically, they are rather thin-walled and do not possess the proteinaceous coating observed on ascarid eggs. Transmission is largely based on contamination of items in stalls or paddocks, where horses scratch themselves to relieve itching in the perineal area caused by the egg deposits. Eggs therefore can adhere to stall or paddock items and horses become infected through accidental ingestion. Human pinworm eggs have been shown to tolerate freezing, but they are more susceptible to warmer temperatures (Caldwell 1982). It is generally perceived that pinworm eggs can survive for several weeks in the environment.

Tapeworms

Tapeworms all have an indirect life cycle which includes an intermediate host. *Anoplocephala* and *Anoplocephaloides* species infecting horses use oribatid mites as intermediate hosts, and the horse becomes infected by accidentally ingesting the mites while grazing. Pasture infectivity, therefore, is essentially a function of mite populations and their ability to withstand various environmental factors. Although mites can survive cold winters to some extent, they are only active during the grazing season. Therefore, tapeworm burdens tend to accumulate through the grazing season and peak during autumn in northern temperate climates. Numerous species of oribatid mites have been shown to serve as intermediate hosts for anoplocephalid cestodes, so it is difficult to propose general rules for their epidemiology. However, given the ability of mites to survive winters, the potential for overwintering pasture infectivity of tapeworms is probably similar to that of strongyles and ascarids.

Conclusion

Generally speaking, the gastrointestinal parasites of horses have adapted their life cycles to function within a life span of 1 year. Horse pastures cannot be expected to remain infective for more than a maximum of 1 year, almost regardless of the pasture type. Thus, parasite burdens do not accumulate during successive years. A horse pasture that has been grazed for 20 years contains no more strongylid larvae than one used for a single season. *P. equorum* appears to be the exception to this rule because of the longevity of its eggs. Regardless, even this nematode seems to have adopted a year-to-year transmission pattern.

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4 Host Factors Affecting Parasite Transmission

Every host–parasite relationship involves countless interactions which affect transmission. These range from mortal combat to apparent collaborations, and occur from the molecular to the population level. In a successful and sustainable relationship, the host provides shelter and sustenance so a resident population of worms can reproduce, and the tenants refrain from destroying their domicile. Vandalism is optional.

In any population of grazing horses, the distribution of parasites among the members of a herd will always be nonuniform and skewed. Some individuals will have large worm burdens, whereas the majority harbor small or moderate numbers. This pattern is often referred to as the 20/80 rule, meaning that 20% of the horses harbor 80% of the parasites in any given herd. The same general pattern is observed in the levels of egg shedding within a herd (Kaplan & Nielsen 2010). As a consequence, parasite transmission is always dominated by a minority of the horses. These egg-shedding patterns are the key to understanding and controlling parasite transmission. The reasons why some horses shed high numbers of eggs, and others very low numbers, are not well understood, but a few factors can be identified with certainty.

Immunity

Host immunity plays a major role in limiting the transmission of nearly all parasitisms, but equids host two examples that are unique among grazing animals for their totality: *Parascaris equorum* and *Strongyloides westeri*. It is, respectively, uncommon and extremely rare to encounter adult horses with patent infections of these nematodes. In comparison, horses of all ages can be infected with strongyles, but foals and juveniles generally exhibit higher egg counts than adult horses. Observational studies have demonstrated that strongyle worm burdens tend to remain fairly constant in horses as they age, but the corresponding egg counts decline chronologically (Chapman *et al.* 2003). This suggests that the main effect of immunity is not to prevent parasite establishment, but rather to control the fecundity of female worms and thereby affect transmission.

Other studies have shown that intensive anthelmintic treatment of horses through one grazing season made them more susceptible to challenge infections during the following year (Monahan *et al.* 1997). These findings support the notion that acquired immunity has an operative role in parasite transmission, although horses never become completely immune to strongyles.

Additional observations suggest that certain innate mechanisms may also help the host to regulate transmission. For example, the magnitude of strongyle egg shedding by adult horses is strikingly consistent over time, and is particularly pronounced in horses that pass low numbers of strongyle eggs (i.e., <200 EPG) (Nielsen *et al.* 2006; Becher *et al.* 2010). The effector mechanisms of this phenomenon remain unclear, but a genetic component is likely to be involved. If so, it is expected that various blood lines within a breed would exhibit patterns of egg shedding that differ significantly from the average. This hypothesis is supported by findings in ruminants, where resilience to parasite infection has been shown to be hereditary (Stear *et al.* 1984; Gasbarre *et al.* 1990). However, similar studies have not yet been performed with horses.

Grazing behavior

Pastures grazed by horses develop a distinct pattern of usage, characterized by areas known as “roughs” and “lawns.” Roughs consist of large or small islets of unconsumed forage, and are areas of pasture where horses defecate but do not graze. In contrast, lawns are areas where horses graze, but do not defecate. The herbage of lawns can become quite short in overstocked pastures. Horses have an aversion for ingesting forage in proximity to fecal deposits, so these spatial patterns develop as a consequence of fecal avoidance behavior. Grazing cattle behave similarly, although unconsumed forage is distributed focally rather than organized

into large areas. In all grazing animals, fecal avoidance behavior is apparently driven by the sense of smell (Hansen 1982).

The numbers of infective strongyle larvae in roughs have been shown to be 10–15 times higher than in lawns (Herd & Willardson 1985). Thus, selective grazing behavior provides an elegant, natural system by which horses regulate their parasite exposure. As long as pastures are not overgrazed, resident horses can continue to eschew the roughs, thereby diminishing their daily intake of infective larvae. On a pasture with limited herbage, pecking-order interactions may force the lowest-ranking individuals to graze farther into the roughs. Thus, horses grazing the same pasture can have dissimilar parasite exposures, and their resulting parasite burdens are expected to be different. This circumstance may explain, in part, the skewed distribution of parasite populations within a herd of horses.

Stress

The impact of stress on host immunity is well documented (Segerstrom & Miller 2004), although the component mechanisms are highly complex and the physiologic effects can be very difficult to measure. Relevant to the current discussion, strongyle egg counts routinely increase in response to host stress, and demonstration thereof requires no specialized equipment or training (see Chapter 9).

Field observations by one of the authors (MKN) suggest that new arrivals are likely to have significantly higher strongyle egg counts than permanent residents of the farm. The multiple stressors which accompany transfer include adjustment to new premises and diet, interacting with a different microbiotic environment, and establishment within the social dominance hierarchy. The effects of transport and translocation on strongyle egg counts need to be confirmed in larger studies.

Case records from equine referral hospitals indicate that their patients often exhibit high strongyle egg counts. In comparison to recent herd records for the same individual, a health condition requiring hospital admission may be accompanied by tenfold or greater increase in egg counts. Because this expansion occurs over a relatively short time period, it can be assumed that adult worm burdens are relatively unchanged. Thus, the only feasible explanation is increased fecundity of female worms. Stress, therefore, may not affect the size of the worm burden in the short term, but rather the magnitude of egg shedding.

Athletic competition is another well-known source of equine stress because it requires strenuous training and frequent transportation to competitive events. In one pertinent example, Standardbred trotters that had more racing success during a single season exhibited a tendency toward higher strongyle egg counts compared to the remainder of "also-rans" (Fog *et al.* 2011). The performance of winning horses was obviously not impeded by their worm burdens, but the accompanying stress contributed to increased egg output and thus potentiated parasite transmission.

Physiologic stress is accompanied by temporary or sustained elevations of plasma cortisol. Equine Cushing's disease (pituitary *pars intermedia* dysfunction) is a common endocrine disease of middle-aged to geriatric horses which is characterized by excess secretion of adrenocorticotropic hormone and increased production of corticosteroid hormones by the adrenal gland. One recent study reported that horses with Cushing's disease had significantly higher strongyle egg counts than age-matched controls (McFarlane *et al.* 2010). Cushing's horses could possibly have larger worm burdens, but it is more likely that their compromised immune function allows resident parasites to shed a higher numbers of eggs.

Similarly, it is often assumed that geriatric horses (aged 20 years and older) require more intensive parasite control efforts. Any horse may begin to shed higher numbers of strongyle eggs if it experiences dental problems or loses body condition, and these health issues are more common in aged horses. Although one study reported higher egg counts in horses ≥23 years (Dopfer *et al.* 2004), another epidemiologic survey observed no differences in this age group (Osterman Lind *et al.* 1999). In general, the egg counts of a healthy, geriatric horse in good body condition should not be substantially different than historical counts from the same animal when it was middle-aged.

In addition to these examples, studies in non-equid host species have demonstrated that concurrent infection with other infectious agents can lead to higher worm burdens and higher levels of egg shedding (Supali *et al.* 2010). Thus, parasite burdens might require extra attention in horses with concurrent illnesses.

Concluding remarks

Although the mechanisms which regulate parasite transmission are not fully understood, we can at least identify horses which require more anthelmintic treatments than others. It is well known that younger horses always need more attention in parasite control programs. But, in order to control parasite transmission more effectively, it is equally important to consider new arrivals, horses that are subordinate in the herd hierarchy, competitive horses being transported frequently to events, and horses that are unhealthy from nonparasitic causes. Egg-shedding patterns almost always follow the 20/80 rule, and even in the absence of the above-mentioned risk factors, there will always be a few high-shedding horses in every herd. It is therefore important to identify this high-contaminating minority in order to manage the herd optimally.

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5 Parasite Factors Affecting Transmission

Most parasites could be thought of as reincarnated, 17-year-old boys. Their major concerns in life are eating and sex, and not necessarily in that order. In physical form, most nematodes are little more than a cylindrical package of reproductive organs. The digestive tract is a simple tube, so even the basic organs of life support are reduced to a minimum. Egg production is the most critical factor in life for these beasts, and all necessary resources are diverted to that purpose. Apropos to this strategy, female nematodes are often two or more times the size of their male counterparts.

A central tenet of biology is that success for any organism can be defined as propagation of the next generation. And to achieve such success, biological organisms must constantly evolve to adapt to changing environments. Even the simplest parasitic helminths of veterinary interest occupy at least two different habitats during a typical life cycle: the vertebrate host and the external environment where transmission occurs. In comparison to feral host populations, the forces of change are many times greater for parasites in domesticated animals because various management factors (e.g., diet, hygiene, confinement vs. turnout, use of anthelmintics) alter both the host and the environment in terms of parasite survival and reproduction.

This chapter will review some of the parasite factors which affect transmission, that is, successful propagation of the next generation.

Reproduction

Fecundity

A basic survival strategy of many parasitic species is to overwhelm the odds against success by sheer force of numbers. If only 1% of the eggs of a particular species ever successfully develop into a next generation, the odds of survival are obviously greater if many, many eggs are produced.

Fecundity is defined as the average number of reproductive products produced per unit time by a single female organism. The females of some parasitic species are extremely fecund; and the Ascaridoid nematodes are widely considered the champions of this strategy. True to form, females of the sole equine representative of this group, *Parascaris equorum*, can each produce approximately 200,000 eggs/day.

Reliable figures are not available for the average fecundity of other parasites, such as large or small strongyles. Some sources report that adult female pinworms can produce 60,000 eggs in a single event. But, since they often expire after this supreme effort, oviposition by pinworms may be a one-time deal.

Members of the subclass Strongylinae tend to be larger, on average, and are thus likely more fecund than most genera which comprise the Cyathostominae. Estimating the fecundity of cyathostomin species is further complicated by the wide variation in size among the common species, assuming that larger females produce more eggs than the smaller species.

Translation

Translation is defined as the series of changes undergone by a reproductive product (e.g., egg, larva) to render it infective to a host. For instance, freshly passed strongylid eggs are not directly infective to their definitive hosts; they must first hatch and develop to the infective L_3. For the common parasites of horses, most of this development takes place in the environment, as discussed in

greater detail in Chapter 3. In that process, they are subjected to the vagaries of the indigenous climate. One notable exception is the equine pinworm, *Oxyuris equi*, which attaches its eggs to the skin in the host's perianal area. For several days thereafter, the body temperature of the host provides a well-controlled habitat in which the eggs are able to become infective. Through this adaptation, pinworm eggs are able to translate more quickly and with a higher rate of success than if left totally to environmental influences.

Persistence of infective stages

The ultimate strategy of a parasite is to introduce an infective stage into the body of the definitive host through the preferred, successful route. The odds increase significantly if infective stages can be presented to the host over a prolonged period of time. Thus, those parasites with extremely persistent infective stages have an evolutionary advantage for parasitologic success. The grand champion for horses in this regard is *P. equorum* because larvated ascarid eggs are reputed to survive in the environment and to retain their infectivity for up to a decade. The persistence of infective stages of other helminths ranges from weeks to months, with marked variation determined by climatic conditions.

Transmission

Transmission is the entrance of an infective stage into the body of the intended host. Most helminth parasites of equids are transmitted via inadvertent ingestion, which is an appropriate strategy for a definitive host that is an obligate grazer. Parasitic infection is admittedly a haphazard consequence of the grazing lifestyle, but some parasites have adapted specific strategies to increase the likelihood of transmission.

One example is the proteinaceous coating of *P. equorum* eggs. This covering helps to protect eggs from environmental conditions, but it may also enhance their ability to adhere to fomites in the environment, including the vertical walls of stalls, and the udder of a mare. The latter site virtually ensures infection of foals during the process of suckling, and the former exploits the characteristic behavior of foals to explore their environs orally. Similarly, the passage of *Oxyuris equi* eggs in clumps virtually ensures that any accidental encounter by a susceptible host will result in multiple, simultaneous exposures.

Adult *Gasterophilus* flies attach their eggs to specific sites on the haircoat of the horse. Some species deposit their eggs in a location from which they can migrate into the oral cavity. For example, *G. nasalis* oviposits in the intermandibular area and hatching first instars make their way through the commissures of the lips and into the mouth. Others lay their eggs in locations that are accessible to oral ingestion by the host parasite or herd mates; oviposition by *G. intestinalis* females on the lower limbs is a well-known example. Grooming by the horse (specifically, the carbon dioxide and high humidity of exhaled air cause eggs to hatch) facilitates direct introduction of first instars into the oral cavity. But, *G. intestinalis* eggs are often deposited on the mane and withers as well, areas that clearly cannot be accessed by the primary host. However, mutual grooming by herd mates targets these particular areas, so the instars are successfully transferred to an equid host, albeit not the one on which the eggs were initially deposited.

Another exceptional adaptation for transmission is seen in *Strongyloides westeri*, which can maintain a free-living life cycle for several generations in the total absence of equid hosts. *Strongyloides* is also unique in that infection may be acquired percutaneously, so oral ingestion is not required.

Filarioid nematodes live within tissues or occupy host sites which have no direct connection with the external environment, so transmission is problematic. However, most filarioids exploit hematophagous arthropods, not only as a means for escaping the host, but also as the preferred site for essential development in the environment. Thus, *Onchocerca* microfilariae migrate to the skin, where they are ingested by certain biting flies, and these arthropods ultimately transfer the nematode into a new host via their feeding activities.

Finally, the parasitologic literature reports many examples of intermediate hosts whose behavior is modified by the infective parasitic stages which they harbor (Chubb *et al.* 2009). Such altered behaviors inevitably increase the likelihood that the

intermediate host will be consumed by a definitive host, thus promoting propagation of the next parasitic generation. All cestode parasites of horses are transmitted through ingestion of soil mites of the family Oribatidae. Oribatid mites are mobile, but there is no evidence that the behavior of mites infected with cysticercoids is altered in any fashion that would ultimately favor parasitic transmission.

Persistence of adult parasites

The scientific literature contains remarkably little information about the longevity of various internal parasite species. Prior to the application of radiolabeled molecules, it was impossible to track the fate of individual worms within a population. It is even problematic to monitor the characteristics of an entire population unless the host is maintained under circumstances which obviate reinfection.

There is little advantage for a parasite to hang around once it has accomplished its major objective and contributed to the propagation of a new generation. Accordingly, most species demonstrate a spike in reproductive activity soon after reaching maturity, and egg laying diminishes steadily thereafter over time. The inevitable decline in fecundity may be due to senescence of the parasite, but host immunity also serves to limit egg production in some host/parasite systems.

From an evolutionary standpoint, it would make sense for parasites to maintain maximal reproductive activity throughout any season in which the environmental conditions for translation and transmission are favorable. In most climates, the maximum duration would be approximately 6 months, but most species do not maintain peak reproductive activity for such a long interval. The average life span for adult large strongyles and cyathostomins, after achieving maturity, is probably no more than 3–4 months (Reinemeyer *et al*. 1986).

Botflies (*Gasterophilus* spp.) are all considered univoltine, meaning they develop only a single generation annually. Of that interval, they spend only a few days as adults, approximately 1 month as eggs, and the remainder as first through third larval instars or pupae.

Little is known about the longevity of equine cestodes, but tapeworms can be some of the most long-lived creatures in the helminth pantheon. It is likely that individual cestodes that are acquired during the grazing season are able to survive through winter and at least until the beginning of the subsequent grazing cycle.

Adult filarioids are known to survive for several years in the tissues of the definitive host. This characteristic is apparently exhibited by *Onchocerca*, because dermatologic signs caused by microfilariae tend to recur annually.

Seasonality of reproduction

One thing that distinguishes helminth parasites from other infectious organisms is the requirement for reproductive products to return to the environment (i.e., outside the host) where they must undergo some essential change before becoming infective to another host. Thus, it is only logical that equine parasites would have evolved to exploit these environmental limitations to the fullest. The single best example of this exploitation is synchronization of their reproductive activities (i.e., egg laying) with the seasonality of environmental conditions that are suitable for translation and transmission.

Ascarids

With their extremely persistent infective stages, ascarids would not seem to need a seasonal strategy for transmission. However, seasonal transmission (in northern climates) occurs by default due to the seasonality of foaling and the inevitable acquisition of effective host immunity. Thus, the majority of ascarid infections are seen in suckling foals during late spring and summer, and in

weanlings and yearlings through autumn and winter, respectively.

Large strongyles

The acquisition of larval infections during the grazing season, combined with the relatively long prepatent periods of the large strongyles, generally result in the onset of patency several months later, usually near the start of a new grazing season. The long prepatent period (PPP) of large strongyles may have been an evolutionary adaptation to inefficient reproductive activities (e.g., producing eggs that end up in a snow drift), and synchronizing patency with climatic conditions that were favorable for egg hatching and development of larvae.

From the standpoint of practical control, the prolonged life cycles of large strongyles makes them particularly susceptible to control and even eradication efforts. This will be covered in greater detail in Chapters 7 and 11.

Cyathostomins

The life cycles of cyathostomins, at least when they develop progressively, are much shorter than those of the large strongyles. When arrested development is implemented, however, their total times of residence might be two to three times longer. New larval infections are acquired during the grazing season, and a portion of the newly acquired larvae mature quickly and begin to lay eggs later in the same grazing season. This population may be responsible for the so-called "summer rise" of strongylid egg counts reported by Herd in 1986. However, other members of the population may undergo prolonged arrested development as early third-stage larvae (EL_3) in the mucosa of the cecum or ventral colon. Eventually, they develop to the late third larval stage (LL_3) and L_4 within fibrous cysts in the gut mucosa, and total residence in the host before maturity may exceed 2 years (Gibson 1953).

Ultimately, worms emerge from the mucosal cysts and begin to reproduce; this often occurs in synchrony with the onset of the local grazing season. Thus, in northern temperate climates, we see the so-called "spring rise" in strongylid egg counts from March to May (Poynter 1954; Duncan 1974). This phenomenon serves to contaminate the environment just when environmental conditions virtually ensure successful development of infective larvae. In feral, free-ranging herds, this also coincides with the availability of nonimmune, susceptible juveniles that are beginning to practice grazing as an essential means of acquiring nutrients.

The prolonged arrested development of cyathostomes within gut tissues is unparalleled among nematode parasites of domestic animals. Certain nematodes can become arrested in various somatic tissues, such as *Toxocara canis* or *Ancylostoma caninum* in visceral tissues of dogs, *Trichinella spiralis* in the muscle tissues of virtually any intermediate host, and even *Strongyloides westeri* in the ventral abdominal wall of a mare. However, no other species has arrested stages which remain in the bowel for longer than a single annual cycle. The persistence of populations in arrested development for greater than two grazing seasons must be a response to certain evolutionary pressures, but the exact nature of those selection factors remains speculative. Such an adaptation would be logical if environmental conditions favorable for translation were ephemeral or even absent from one season to the next. It would also make sense if prehistoric equids were nomadic, but returned to preferred grazing areas at intervals that were greater than once annually. Since larvae in the environment cannot survive for much longer than 1 year, prolonged arrest provides a mechanism by which reproducing populations could be ensured, and eggs for environmental contamination would be available to infect the new pasturage and ensure transmission within just a few weeks.

It should be noted here that encysted stages of cyathostomins are not uniformly susceptible to any anthelmintic regimen, no matter how heroic. Thus, if an infected horse were treated very intensively, even with larvicidal anthelmintics, its strongylid egg counts would soon be reduced to zero and would remain there for several weeks or months. Eventually, however, cyathostomin eggs would reappear in the feces, even if the horse had been housed in sterile conditions. From a control standpoint, horses will always take their cyathostomin populations with them to a new environment. Thus, the so-called "treat and move" strategies that worked so well for control of ruminant parasites were never quite as successful in horses. In summary, eradication of cyathostomins is neither feasible nor desirable.

Bots

The adult stages of stomach bots are flies (dipterans) which share a common seasonality with most other free-living arthropods. Thus, eggs are deposited on the haircoat of the host during late spring through autumn, but fly activity ceases, as legend would have it, with the occurrence of regular frosts in late autumn in temperate areas. Bot larvae overwinter within the host as a strategy for avoiding harsh climatic conditions in the environment. Due to seasonality, parasitic bot stages (larvae in the oral cavity and alimentary tract) are present during summer and autumn, and acquisition continues until oviposition is terminated.

From a control standpoint, single treatments are most effective if not administered until after oviposition by adult stages has ceased, usually in late autumn of each year.

Adaptation to control efforts

Any genetically based adaptation to control efforts is an attempt to survive and propagate another generation. Although anthelmintic-resistant nematodes survive treatment to persist as individuals, it is of greater evolutionary significance that their reproductive efficiency remains virtually unimpaired.

Certain parasitologic irregularities might represent yet other biological efforts to counteract control attempts. For example, both ascarids and pinworms were traditionally considered parasites of juvenile horses, and their occurrence in mature animals was worthy of comment. In recent years, however, many practitioners have documented both patent ascarid infections and problematic pinworm infections in adult horses. One feasible explanation for these atypical observations is that the nematode - populations are simply adapting, out of necessity, to a broader array of hosts. In feral horse bands, a significant portion of the entire population would be juveniles year after year. So, access of pinworms and ascarids to an appropriate class of host is ensured. In confined herds, however, reproduction is managed, and juveniles may disappear entirely from the herd structure on some farms. In managed herds which still include juveniles, the younger horses are often the focus of intensive deworming efforts. So, nontraditional sources of refuge, such as adult horses, would be advantageous in either case.

Practitioners must begin to consider not just the positive benefits of parasite control efforts (decreased contamination, lower worm burdens, increased productivity), but also the potential costs in terms of selection pressure and limited biological diversity. When challenged so intensively, parasites must either adapt or die, and Mother Nature abhors extinction.

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Section II

Principles of Equine Parasite Control

6 Decreasing Parasite Transmission by Nonchemical Means

In order to standardize the concepts discussed in the following chapter, let us introduce some relevant technical terms which are not interchangeable.

Definitions

Contamination: The introduction of reproductive products (e.g., strongyle eggs) into the environment. Contamination is initiated by the host, and for most equine parasites, is implemented through defecation.

Translation: A series of events which transform a reproductive product (e.g., a strongyle egg) into an infective stage (e.g., third-stage larva). For most nematode parasites of any importance to equids, translation invariably occurs outside the host in the environment.

Infectivity: The availability of infective stages in the environment. For equine strongyles, for instance, this equates to infective, third-stage larvae. Infectivity is a quality of the environment, not the host, and can be described quantitatively, for example, numbers of third-stage larvae per unit of pasture area or weight of forage. Infectivity represents the risk of infection.

Introduction

Parasitism is a progressive and cyclic process, so it is logical that parasite transmission can be confounded, and future generations prevented, by blocking any single event during the course of a life cycle. Most control efforts to date have focused on those portions of the life cycle occurring within the host, and have relied almost exclusively on administration of anthelmintics. In order to disrupt transmission effectively, the chemical tools currently at our disposal must be implemented at some point between infection (i.e., ingestion of infective stages) and patency (i.e., passage of reproductive stages). However, parasite transmission can also be hindered by various nonchemical strategies directed at the host, parasite, environment, or combinations thereof.

Measures to limit contamination

The major objective of most parasite control efforts is to prevent contamination of the environment with reproductive products of the respective parasites. Once eggs enter the environment (termed “contamination”), further development is almost totally under the control of ambient conditions. Passage of feces which contain parasite eggs carries the threat of imminent infection if contemporary environmental conditions are favorable for translation (hatching of eggs and development into infective stages). Conversely, environmental contamination has essentially no parasitologic consequence during seasons when climatic conditions are not favorable for translation, or if eggs are deposited in an unsuitable habitat. Whenever the climate or habitat does not support translation, fecal contamination is entirely permissible from a control standpoint.

Although contamination is the last chronological step in the life cycle at which traditional control methods can be implemented, this limitation should not discourage us from theorizing about future approaches with the same objective. For example, a birth-control product that sterilized male or female nematodes would provide extremely effective control, even if it did

not kill the adult parasites, because it would reduce environmental contamination with fertile eggs. The following discussions expand on various practical or theoretical ways in which we could decrease contamination.

Ensuring that feces contain few eggs

Dangerous levels of infectivity do not result when horses with relatively low fecal egg counts defecate on pasture. Horses with fecal egg counts lower than 100–200 eggs/g probably have little impact on pasture infectivity as a whole, even when conditions are favorable for translation (see Chapter 7).

Three conditions explain why the feces of a specific horse might contain relatively few strongyle eggs. The first is that the horse is a low contaminator, and its minimal egg counts are the manifestation of a permanent genetic trait. Such low egg counts generally persist for that horse's lifetime, even in the absence of anthelmintic treatment. Accordingly, low contaminators may be given access to pastures at virtually any time and season without negative implications for the remainder of the herd.

The second reason for a low fecal egg count is historical exposure to light levels of infectivity. Nematode parasitism is a quantitative phenomenon, and large numbers of worms cannot develop unless the host was previously exposed to high numbers of infective larvae. Horses in this category most often originate within management systems which limit exposure to infectivity (e.g., constant confinement), feature very low stocking densities (e.g., one horse per 5 acres), or occur in regional climates which are not conducive to strongylid translation (e.g., the arid southwestern U.S.).

The third explanation for low fecal egg counts is recent anthelmintic therapy. Treatment with any effective dewormer (by definition >95% FECR [fecal egg count reduction]) reliably reduces fecal egg counts for a predictable interval post-treatment. This interval is termed the "egg reappearance period" (ERP; see Chapter 9 for full discussion). For the duration of the predicted ERP, horses treated with the respective anthelmintic will not be sources of significant pasture contamination. However, if the targeted cyathostomins were resistant to the anthelmintic class just administered, fecal egg shedding would continue unabated. Reproduction by resistant individuals increases the genotypic frequency of resistance in the infective larvae available to grazing horses, and accelerates the rate of resistance development within the worm population as a whole.

Controlling where defecation occurs

In some metropolitan areas, equids pulling public conveyances must be outfitted with "diapers" to collect feces and thereby prevent soiling of public streets. Although capturing feces is impractical for routine equine management, this measure would effectively preclude contamination of the environment and thereby totally block transmission of most parasites.

A more feasible management tool for limiting environmental contamination would be to restrict pasture access for high contaminator horses (see Chapter 7) during seasons when climatic conditions are favorable for translation.

Regular removal of feces

The consequences of environmental contamination can be minimized if horses defecate in sites from which feces can be removed easily. Horse manure can be collected most completely when the pellets are still intact, so the feces of confined horses should be removed frequently enough to prevent mechanical disruption by the stall occupant(s). Manure may be collected less frequently from bedded paddocks or dirt lots. These venues pose minimal risk for strongyle transmission because they contain no vegetation to provide a protective microhabitat for larval persistence.

Quarantine practices

Horses arriving on a new farm should be quarantined before being turned out to pasture with other residents. The parasitologic goal of quarantining is to prevent or minimize the introduction of new or genetically unique parasites that differ from the

indigenous population. Examples include a highly pathogenic parasite, such as *Strongylus vulgaris*, which is usually not found in most well-managed herds. *Strongylus vulgaris* is not known to be resistant to any of the drug classes currently available, so virtually any broad-spectrum treatment will remove adults. One should remember, however, that not all anthelmintics are effective against the migrating larval stages of *S. vulgaris* or *S. edentatus*.

Horse owners should be particularly concerned about the inadvertent introduction of drug-resistant parasites. Strains of cyathostomins or *Parascaris equorum* that are resistant to specific drug classes can be imported via newly arrived horses. To quarantine new arrivals with positive fecal egg counts, a pragmatic approach is to treat them with the most commonly used drug on the farm, and then hold them in confinement until a fecal egg count reduction test can be completed 14 days post-treatment. If the result is satisfactory (i.e., ≥95% FECR for most of the drugs, see Chapter 9), the horse can be turned out. If not, it should be treated immediately with another anthelmintic with a different mode of action.

This strategy is far less successful against occult, larval (i.e., nonpatent) infections because one cannot demonstrate their presence, and there is no immediate way to evaluate the efficacy of an anthelmintic treatment. Readers are referred to other sections in this book which address larvicidal therapy of ascarids and encysted cyathostomins.

On many farms, a traditional quarantine program consists of merely keeping a new horse in its stall for a few days after anthelmintic treatment, and then turning it out to pasture. Presumably, the idea is to allow clearance of the eggs of any new strain from the alimentary tract before releasing the horse to a common grazing venue. But, in cases when the target nematodes are resistant to the anthelmintic used, this measure is fatally flawed without rigorous post-treatment monitoring of efficacy.

Pasture hygiene

Pasture hygiene involves removing feces from pastures on a regular basis. If pastures are cleaned thoroughly at intervals shorter than the time it takes for eggs to develop into infective larvae (see Chapter 3), translation can be disrupted before larvae are available to enter the horses. During the warm months of a grazing season, feces need to be collected at intervals shorter than the 7–10 days required for eggs to develop into L_3. During cooler months, the frequency may be decreased. If possible, unscheduled pasture cleaning should be implemented whenever rainfall ≥1 cm is forecast because heavy precipitation mechanically disrupts fecal pellets.

Pasture hygiene is time-consuming and labor-intensive. Fecal removal has been accomplished traditionally with a manure fork or broom and shovel. However, several types of equipment, usually manufactured for maintenance of golf courses, are now available for vacuum-cleaning or mechanical removal of feces from the ground. These units are pulled by tractors or all terrain vehicles and can be extremely efficient. Pasture hygiene is more likely to be implemented on smaller farms with limited land available for grazing. The workload is correspondingly greater on large farms, although top-end breeding or training facilities may have the financial resources to implement this measure, regardless. If implemented meticulously, a pasture hygiene program can nearly eliminate the need for anthelmintic treatments. One study evaluated the effects of removing feces twice weekly using pasture vacuum devices and compared it to anthelmintic treatment regimens. Based on quantitative assessments of infectivity, the pasture hygiene approach yielded an 18-fold reduction in larval numbers, compared to an untreated control group, and was four times more efficient than using anthelmintic treatments alone (Herd 1986a). These same studies determined that mechanical sweeping was a more feasible approach than using a vacuum apparatus (Herd 1986b).

Whether manure is collected from stalls or pastures, it should be composted properly to minimize any parasitologic risk. As described in Chapter 3, strongyle larvae and eggs cannot survive temperatures in excess of 40°C. Effective composting can generate temperatures of 70°C, and should effectively kill parasite products within the manure. However, thorough composting requires frequent turning of the organic material. If left unturned, a compost heap develops temperature gradients in different strata, and conditions in the exterior layers may be favorable for parasite development. Little is known about the thermal tolerance of *P. equorum* and *Anoplocephala* eggs, but they would likely be killed by the high temperatures resulting from effective composting.

A potential disadvantage of fecal removal is that the pasture ecosystem loses valuable nutrients, and supplemental fertilization may be required. However, after thorough composting, manure can be spread onto pastures with no attendant risk of parasite transmission.

Measures to limit infectivity

Stocking density

Stocking density is the single most important factor affecting parasite transmission. Whenever pastures are overgrazed, no management intervention can be optimally beneficial. Feral, nomadic horses presumably experienced relatively little parasitic disease, but once horses were domesticated and confined within artificial barriers, they could no longer avoid their nematode visitors.

Proper stocking density is difficult to define because it depends on numerous factors such as soil type, variety and quality of the herbage, local precipitation, size and nutritional requirements of the horses, etc. As a rule of thumb, one horse per 1–2 acres of land should not result in overgrazing, but the best way to assess stocking rate is to evaluate the pasture. Under most conditions, forage in the lawns should be no shorter than 5–7 cm (2–3 in.), and the roughs, where horses defecate, should remain ungrazed.

Regulating stocking rates is often difficult to achieve because both herd size and grazing acreage are finite numbers. The latter cannot be increased easily, and human nature tends to expand, rather than diminish, herd size over time. Renting extra land or sending horses away for summer grazing are helpful options, if available. A short-term remedy is to supplement the feed of horses on pasture. As long as forage is not the sole source of nutrients, overgrazing can be delayed, if not prevented. Pasture hygiene is the most efficient solution for parasite control on farms where overstocking and overgrazing are a problem.

Harrowing or mowing pastures

Horses are notoriously hard on pastures. Their hard, sharp hooves cut the turf and compact the soil. In addition, equine fecal piles are more prominent than those of any other grazing species, and the tendency of horses to divide pastures into roughs and lawns results in irregular and overgrown areas. Consequently, most horse owners harrow, or "drag," their pastures to break up and distribute fecal piles, and mow down roughs and tall weeds to create a more uniform appearance. These common management practices can have unintended consequences.

Harrowing is widely believed to reduce parasite transmission because intact fecal pellets provide a protective habitat for strongyle eggs and larvae. Breaking up fecal pellets or disseminating fecal piles over a large area exposes free-living stages to unfavorable environmental conditions. However, distributing feces uniformly over an entire pasture confounds the selective grazing behavior of horses described in Chapter 4, and exposes horses to greater levels of infectivity. Similarly, roughs are foci of retained moisture, which is favorable for larval translation. Mowing roughs and tall weeds contributes to the desiccation of pasture herbage, and is widely believed to reduce larval survival. However, it should be noted that strongyle L_3 have been shown to be quite resistant to desiccation (Chapter 3).

It is recommended that horses be removed from any pastures that are being dragged or mowed, but the obvious question is how long the pasture must be rested. The recommended rest period varies with geographic location, climate, season, recent weather, types of forage, stocking density, soil types, etc. In temperate climates with warm but not hot summers, such as northern Europe, northern U.S. and Canada, temperatures and moisture levels are likely to favor survival of strongyle L_3 for months. Therefore, mowing or dragging can only be recommended for pastures that will not be grazed again within the same season. In tropical and subtropical climates, summer temperatures are often too hot to support significant parasite transmission. Subtropical weather conditions are more favorable for parasite transmission during autumn, winter, and spring, when considerations for and against mowing and dragging pastures are similar to those during summers in northern temperate climates. In hot, arid regions, however, L_3 can survive for some time within intact fecal pellets, and then migrate onto forage after heavy rainfall. Mechanical disruption of fecal pellets during dry periods would reduce larval survival in arid regions.

It is interesting to note that studies performed in the 1930s showed that in areas with cold and snowy winters, dragging pastures at the end of the grazing season markedly reduced winter survival of strongyle larvae (Parnell 1936). Presumably, disrupting fecal pellets rendered larvae more vulnerable to environmental influences.

Pasture rotation

Pasture rotation is performed for various reasons. Rotation may serve as a method for optimizing the use of pastures as a nutrient source. For this strategy, pastures are rested for relatively short intervals, approximately 2–4 weeks to allow herbage regrowth before horses are turned back in.

From a parasitologic standpoint, moving horses from one pasture to another might serve to interrupt the strongyle life cycle and effectively reduce parasite transmission. Timing, however, is the most critical issue. Determining when to reintroduce horses to a pasture grazed earlier in the same season depends on climate and weather. In northern temperate climates, it is unlikely that pasture infectivity will diminish significantly within the same grazing season. As a rule of thumb, a northern pasture needs to be rested until the beginning of the subsequent summer before it can be considered relatively parasite-free. In tropical climates, however, significant reductions of larval counts can be observed after just 2–4 weeks of rest (Barger *et al.* 1994).

In temperate areas, horses could be moved during midsummer to a pasture that was not grazed previously during the same season. Hay aftermath (a pasture from which a crop of hay has been cut earlier in the season) is ideal for this program, and such pastures are often ready for grazing by early July.

Mixed or alternate grazing

Alternating hosts of different species on the same pasture has been shown to reduce parasite transmission. The key principle is to interchange two animal species that do not share the same parasites; thus, alternating cattle and horses would be superior to alternating cattle and sheep. The potential benefits of this practice include: (1) more efficient use of pasture as a nutrient source, (2) termination of the life cycle when L_3 are ingested by a non-suitable host, and (3) consumption of the herbage in roughs reducing survival of free-living stages.

Cograzing cattle and sheep with horses have both been investigated. Mixed grazing reduced parasite transmission, but allowing horses and ruminants simultaneous access to a pasture had the disadvantage of increasing the stocking density. Alternate grazing was generally more effective. However, it should be noted that horses share a few parasites with ruminant species. In one study to investigate the effects of cograzing Shetland ponies and sheep, the prevalence of *Trichostrongylus axei* increased in the ponies, although overall strongylid infection was reduced (Eysker *et al.* 1983, 1986). Similarly, horses can be infected by the liver fluke, *Fasciola hepatica*, and this should be considered in areas where fluke infection is endemic in local ruminants.

Mixed or alternate grazing is implemented only rarely, however, perhaps because most horse owners do not simultaneously own adequate numbers of ruminants to effect a successful program.

Another grazing strategy, termed the dilution principle, has been studied in cattle and sheep. This practice is based on the observation that older, more resilient animals have smaller worm burdens and exhibit lower fecal egg counts than younger, more susceptible individuals. By cograzing old and young stock, the older hosts ingest infective larvae with little consequence, and the younger animals are exposed to progressively lower infectivity (Nansen *et al.* 1990). Extrapolating this strategy to horses would involve keeping adult horses and juveniles together on the same pasture.

Another grazing strategy, termed the leader/follower approach, has been described for ruminants (Leaver 1970). In this system, a pasture is grazed first by a group of young, parasite-susceptible animals (leaders). As expected, these nonimmune hosts develop high egg counts and cause serious pasture contamination. The young animals are removed from pasture before infectivity reaches hazardous levels, and older, immune hosts (followers) are introduced. The followers ingest far greater numbers of larvae than the leaders, but with less impact on health and productivity. The basic principle behind this strategy is to let both age groups utilize forage efficiently, but the older animals have sufficient acquired immunity to deal with a serious larval challenge. The leader/follower approach has not been evaluated in horses. However, large breeding farms would have the resources to allow weanlings or yearlings to graze a certain pasture first, and then follow them with a group of mature horses. Similarly, perhaps a pasture could first be grazed by horses with high egg counts (juveniles or known high contaminators), and then followed by another cohort of low contaminators. Presumably, the latter group would maintain low egg counts despite intensive larval exposure. The leader/follower approach remains purely speculative for horses, however, and has not been evaluated scientifically.

Pasture renovation

Pasture renovation should be performed on a regular basis to ensure good herbage quality and to prevent the dominance of weeds. Plowing, cultivation, reseeding, and fertilization of pastures can also have a significant impact on resident populations of free-living stages of parasites. Strongylid larvae, for instance, not only survive being plowed under the soil to a depth of 30 cm, but they can find their way back to the surface. However, so much energy is expended in this process that larval survival is severely compromised.

Ascarid eggs do not share the same limitations. Because the infective stage remains within the egg, it cannot return to the soil surface when buried by plowing. So, turning the soil may be a very effective control measure for pastures grazed by foals and yearlings. Studies performed with porcine ascarids have demonstrated that eggs buried in the soil remain viable for several years, and are capable of infecting animals when eggs are returned to the surface by subsequently replowing (Mejer 2006). This scenario is similarly feasible for the eggs of *Parascaris equorum*.

Measures to limit translation

Control methods that could be implemented after reproductive products leave the host (i.e., by blocking translation or reducing infectivity) remain the Holy Grail of applied parasite control. Such novel methodologies would constitute highly desirable approaches, particularly if they were sustainable and did not select for genetic adaptations to the management intervention.

Nematode-trapping fungi

Duddingtonia flagrans is a free-living fungus that occurs naturally in herbivore feces. This fungus traps and effectively kills strongyle larvae after they hatch in the feces (Larsen 1999). The spores of this fungus survive passage through the gastrointestinal tract of livestock. Although the potential nematocidal activity of *Duddingtonia* has been demonstrated by numerous *in vitro* studies, its practical utility has been hampered by the need to provide fungal spores in the diet on a daily basis. In addition, the potential reduction of larval numbers has ranged from 30% to 90% (Tavela *et al.* 2011), which may not be sufficient for effective control. Like so many other approaches, *Duddingtonia* offers good *in vitro* promise, but has not yet found utility under standard management conditions.

Fluorescing compounds

Among the various methods that have been investigated for disrupting larval translation, perhaps the most elegant was the inclusion of erythrosin B, a photodynamic dye, in the daily rations of grazing beef cattle. When fed to cattle that were infected with trichostrongylid nematodes, erythrosin B was absorbed by adult female worms and incorporated into the genetic material of their offspring (Healey *et al.* 1992). This "tagged" generation left the host as eggs, and when their larvae hatched, they also contained erythrosin B. It was hypothesized that larvae developing in the environment would be exposed to sunlight, and ultraviolet radiation would cause the erythrosin B to fluoresce and ultimately kill the developing larval stages on pasture.

Incorporation of erythrosin B into infective larvae and inducing its lethal effects worked very well in the laboratory, but was less successful in typical livestock management situations (Hawkins *et al.* 1986). Its utility was hampered by two, basic, biologic factors. First, bovine nematode larvae spend much of their developmental time within the fecal pat, where they are not exposed to sunlight. Second, larvae migrate subsequently into the thatch layer of pasture forage, where there is also relatively little penetration by ultraviolet light. Regardless of the difficulties with implementation, this general approach is fascinating.

In the future, perhaps lethal genes that are activated by environmental triggers can be incorporated into certain worm isolates as a means to effect translational control. However, equine practitioners must recognize that such unique technologies will first be

developed to manage the potentially lethal nematodes of sheep, and that the relatively benign parasitisms of horses must wait in line behind the more dramatic problems of small ruminants and cattle.

Conclusion

Although this chapter discussed multiple, nonchemical approaches to decreasing environmental contamination, the only one that is implemented with any frequency is frequent removal and composting of feces. Other measures could be adopted as well, with minor investments of time and equipment. The main thrust is to reduce reliance on chemicals as the sole control element used on many premises.

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7 Pharmaceutical Approaches to Parasite Control

In the not-too-distant past, various natural products and processed derivatives were administered to horses in hopes of achieving parasite control. These "remedies" included black soup, calomel, aniseed, aloes, antimony, licorice, linseed, and quicksilver (reviewed by Lyons *et al.* 1999). One imagines that in many cases the cure was worse than the disease. The first historical example of an antiparasitic drug with convincing activity against an equine parasite was carbon disulfide, which was shown to be effective against bots (Hall 1917). Oil of chenopodium was also found to be efficacious against strongyles (Hall *et al.* 1918), but it allegedly had severe side effects such as anorexia and weight loss (Lyons *et al.* 1999). The first modern dewormer launched for equine usage was phenothiazine in the 1940s, which was followed by piperazine in the 1950s (Lyons *et al.* 1999).

Equine parasite control was revolutionized in the early 1960s when the benzimidazole (BZD) drug class was introduced to market. The launch of these inaugural, modern anthelmintic compounds made it feasible, for the first time in history, to disrupt nematode life cycles and prevent parasite transmission. Scientists, veterinarians, and horse owners began to discuss eradication of parasites as if it were a realistic and desirable goal.

Leading equine parasitologists at the time advocated that life cycle features and transmission patterns be considered to make optimal use of these new anthelmintic drugs. They had observed that it took about 2 months for strongyle eggs to reappear after deworming with thiabendazole, and recommended that subsequent treatments should be timed accordingly (Drudge & Lyons 1966). This historical recommendation was the first instance in which an egg reappearance period (ERP) was considered as a parameter in equine parasite control. (See Chapter 13 for a detailed definition of ERP and its implications.) A comprehensive control regimen was devised which featured anthelmintic treatments administered at bimonthly intervals all year long. This regimen was later termed the interval-dose program, and it was rapidly adopted at horse establishments worldwide.

The primary parasitic target for the interval-dose program was *Strongylus vulgaris*, which was highly prevalent at the time and widely considered the major parasitic pathogen of horses. The interval program was highly successful in reducing the prevalence of *S. vulgaris* and associated clinical conditions, but its efficacy was ultimately compromised by the development of anthelmintic resistance in populations of cyathostomins. Anthelmintic resistance was first reported in the 1960s, and it is now recognized in all the drug classes currently labeled as parasiticides for horses.

This chapter provides a brief discussion of the anthelmintic drug classes currently available for treatment of equine parasites, and outlines different principles of anthelmintic treatment. In addition, the chapter includes an extensive discussion of anthelmintic resistance.

Anthelmintic drug classes

The number of pharmacologically unique anthelmintic classes for use in horses is currently very small. In fact, only three drug classes are labeled for efficacy against nematodes, and only two classes for treatment of cestode infections. Relatively recently, the pharmaceutical industry has developed new anthelmintic classes for non-equid animals, but it has been more than 25 years since a nematocidal drug with a new mode of action was introduced for use in horses. In the following section, the various drug classes are presented in chronological order of their entry to market.

Benzimidazoles

The mode of activity of the BZD drug class is to disrupt energy metabolism at the cellular level. BZDs bind to the protein tubulin and block its polymerization into microtubules. Microtubules are essential structural components of many cellular structures, such as telomeres and cilia. These structures are critical to energy metabolism, and BZDs are the only currently marketed anthelmintic class that has a primary, antimetabolic mode of activity. Nematodes have very limited organs of energy storage, so

they must consume nutrients constantly. By blocking energy metabolism, even temporarily, affected worms basically starve to death, and the stages in the intestinal lumen are expelled over a number of days following treatment.

Historically, thiabendazole, cambendazole, fenbendazole (FBZ), oxfendazole, oxibendazole, mebendazole, and the pro-BZD febantel have all been approved for equine use. Of these, only FBZ and oxibendazole are still marketed in the U.S. for horses.

FBZ was the first broad spectrum, equine anthelmintic. For horse anthelmintics, the term "broad spectrum" indicates acceptable efficacy against four target parasites: large strongyles, cyathostomins, *Parascaris*, and *Oxyuris*. When administered at an elevated dosage (10 mg/kg) for 5 consecutive days, FBZ exhibits high efficacy against migrating ascarid and large strongyle larvae, as well as against encysted cyathostomin larvae.

Cyathostomins were the first parasites to demonstrate resistance to a BZD drug. A survey conducted within the past decade found that 95% of the farms evaluated had indigenous populations of small strongyles that were BZD-resistant (Kaplan *et al.* 2004). So today, it appears that BZD resistance in cyathostomins is the rule rather than the exception in managed horses. Although the 5-day regimen of FBZ may initially have satisfactory efficacy against cyathostomin populations with moderate levels of resistance, recent work suggests that the 5-day regimen has little or no effect against highly resistant worms (Lyons & Tolliver 2003; Rossano *et al.* 2010). This confirms the tenet that anthelmintic resistance cannot be overwhelmed by increasing the dosage or the frequency of treatment.

BZD resistance has never been reported in large strongyles or *Parascaris equorum*, so this drug class may still be used with confidence against these target nematodes. The BZD group can still be viewed as very useful, albeit against a narrower spectrum of parasites than when it was first marketed.

Pyrimidines

The pyrimidines (tetrahydropyrimidines) were first introduced in the 1970s and have been used widely ever since. The pyrimidines that are currently available for horses include pyrantel pamoate (international), pyrantel tartrate (North America), pyrantel embonate (Europe), and morantel tartrate, which is labeled for equine use in Australia. Members of this class act as selective acetylcholine agonists and cause rapid, spastic paralysis of nematodes. Paralyzed worms are unable to conduct coordinated feeding activities and would ultimately starve if not expelled by intestinal peristalsis.

Pyrimidines are effective only against luminal stages because there is no parenteral uptake of the drug. Interestingly, pyrimidines are not labeled for efficacy against fourth-stage cyathostomin larvae, and this stage has been shown to survive treatment (Reinemeyer 2003). Generally, pyrantel pamoate (embonate) is available as a suspension or paste product in most of the world, whereas pyrantel tartrate is manufactured in a pelleted formulation and sold as a daily feed additive in North America only. Morantel tartrate is available as both paste and granule formulations in Australia. The pyrimidines are considered to be broad spectrum (large strongyles, cyathostomins, ascarids, pinworms), but also have good efficacy against the tapeworm *Anoplocephala perfoliata*. The label dosage (6.6 mg/kg) often affords better than 80% cestocidal efficacy (Lyons *et al.* 1988), and the activity of 13.2 mg/kg is greater than 95% (Reinemeyer *et al.* 2006).

Resistance to pyrimidine anthelmintics has been detected in some cyathostomin populations, but it is not yet as widespread as for BZDs. Resistance appears to be most pronounced in North America, where 40% of farms were affected in one survey (Kaplan *et al.* 2004). The pyrimidine class works satisfactorily on the majority of farms in Europe, but a low prevalence of pyrantel resistance in cyathostomins has been reported there (Traversa *et al.* 2009). Morantel tartrate-resistant small strongyles have been reported in Australia (Rolfe *et al.* 1998).

Most recent studies have observed good efficacy of pyrantel salts against *Parascaris equorum* (Lind & Christensson 2009, Veronesi *et al.* 2009, Reinemeyer *et al.* 2010). However, one report suggests that some populations of ascarids in the U.S. are resistant to pyrantel (Lyons *et al.* 2008b).

Macrocyclic lactones

Ivermectin was introduced in the early 1980s and was the first representative of the macrocyclic lactone group. This drug class is very broad and comprises both antibiotics and antiparasitics. Therefore, those subgroups of macrocyclic lactones with antiparasitic properties are more precisely termed avermectins and milbemycins (Sangster 1999). For equine use, the avermectin group is represented by ivermectin and abamectin, and the milbemycin group by moxidectin.

Avermectins/milbemycins are known to interfere with the function of glutamate-gated chloride (GluCl) channels, resulting in flaccid paralysis. Paralyzed parasites are unable to ingest nutrients, and those dwelling in the gastrointestinal lumen are expelled by peristalsis. Migrating stages are killed by cellular immune responses. The onset of activity is rapid, like the pyrimidines, occurring within the first 48 h post-treatment.

Avermectins and milbemycins share many properties, with a few important exceptions. These drugs are termed endectocides, meaning they kill internal (endo-) as well as external (ecto-) parasites, because both exhibit activity against nematodes and arthropods. In addition to killing all luminal stages of nematodes, ivermectin and moxidectin are very effective against migrating larval stages, such as those of large strongyles, ascarids, and *Strongyloides*. Efficacy against arthropods includes *Gasterophilus* larvae attached to the walls of the stomach and duodenum (Reinemeyer *et al.* 2000), and ivermectin is labeled for activity against earlier stages residing in the oral cavity. Both drugs have high efficacy against microfilariae of *Onchocerca* spp. in horses (Mancebo *et al.* 1997; Monahan *et al.* 1995). The adult worms residing in deep connective tissues are not killed by macrocyclic lactones, but apparently are rendered infertile for intervals of several months. Compounds of this class are also effective against larvae of the spirurid nematodes *Habronema* and *Draschia*, which cause granulomatous, cutaneous lesions. Neither compound has any efficacy against tapeworms.

Moxidectin was introduced in the mid-1990s and is more lipophilic than ivermectin. Moxidectin accumulates in fatty tissues, which serve as a depot from which the drug is released gradually over time. Consequently, moxidectin differs from ivermectin in two important ways. First, moxidectin demonstrates efficacy against encysted cyathostomins, with activity ranging between 60 and 80% in various studies (Xiao *et al.* 1994). Accordingly, moxidectin is labeled for larvicidal efficacy. Secondly, moxidectin suppresses strongyle egg counts for 12–16 weeks post-treatment (Jacobs *et al.* 1995). This is the longest ERP of any anthelmintic approved for horses, and ~1.5 times that of ivermectin.

Although ivermectin has been the most widely used equine anthelmintic in recent decades, no signs of resistance were reported during its first 20 years on the market. This stellar record was interrupted at the beginning of this century, however, by the first reports of ivermectin- and moxidectin resistant *Parascaris equorum* (Boersema *et al.* 2002; Hearn & Peregrine 2003). Numerous studies have since confirmed the presence of ivermectin and moxidectin resistance in indigenous populations of *P. equorum* on most continents.

Ivermectin and moxidectin still exhibit good efficacy against cyathostomins at 14 days post-treatment, and no signs of resistance have been observed among large strongyles. However, several studies have documented that the strongyle ERPs have become shorter after treatment with either compound. Whereas ivermectin and moxidectin historically suppressed egg shedding for 8 and 12–16 weeks, respectively, recent studies have reported ERPs of 4–5 weeks for both drugs (Lyons *et al.* 2008a, 2011). It is certain that frequent and exclusive avermectin/milbemycin treatment over the years has selected for populations of cyathostomins with shorter ERPs. But, these same observations have been interpreted as early signs of resistance, especially since fourth-stage larvae (L_4) dwelling in the intestinal lumen have been shown to survive treatment at label dosages (Lyons *et al.* 2009, 2011).

Piperazine

Horses were commonly treated with piperazine adipate several decades ago, but the drug is no longer sold for equine use in most countries. Piperazine works as a gamma-aminobutyric acid (GABA) agonist and evokes spastic paralysis. Piperazine demonstrated good efficacy against *Parascaris equorum* and cyathostomins, but not against large strongyles, pinworms, or bots (Drudge & Lyons 1986). Historically, piperazine was largely employed as a co-treatment with some BZDs that had little or only modest efficacy against ascarids (e.g., thiabendazole, mebendazole). Arguably, piperazine might enjoy similar use today when used with a BZD against BZD-resistant cyathostomins or with a macrocyclic lactone against ML-resistant ascarids. Piperazine's biggest drawbacks are a high dosage (110 mg/kg) and relatively dilute formulations which require nasogastric intubation for administration of voluminous doses. Piperazine resistance has been reported in cyathostomins (Drudge *et al.* 1988).

Praziquantel

Praziquantel (PRZ) is a member of the quinolone-pyrazine drug group, and its only application in horses is against tapeworms. PRZ acts by damaging the tegument of the parasite and by modulating cell membrane permeability, which results in spastic paralysis.

PRZ has been used in dogs and cats for decades but was approved for horses only relatively recently. To date, PRZ resistance in equine tapeworms has not been reported, but resistance has been observed in schistosomes (Fallon & Doenhoff 1994).

Other drug classes

During recent years, three new drug classes have been developed for treatment of nematode infections of domestic animals. To date, none of these has been marketed for use in horses. It is largely unknown whether they would be effective against equine parasites, safe for horses, or economically feasible to manufacture. A brief account of each new drug class follows.

Emodepside

Emodepside belongs to the cyclo-octadepsipeptide class and causes paralysis in nematodes by stimulating latrophilin receptors. Emodepside is approved for use in dogs and cats only, and is primarily effective against hookworms and ascarids.

Derquantel

Derquantel belongs to the spiroindole group, and works as an acetylcholine antagonist, causing flaccid paralysis. It is currently marketed in combination with abamectin for use in sheep in New Zealand and Australia, but is likely to be available in other countries in the near future. Derquantel has good efficacy against major trichostrongylid nematodes of sheep. Unfortunately, initial studies have found the drug to be toxic to horses, so it will not be developed as an equine drug.

Monepantel

Monepantel is an amino-acetonitrile derivative (AAD) and acts on a specific acetylcholine receptor subunit in nematodes to elicit paralysis. It is broad spectrum, with high efficacy against major gastrointestinal parasites of sheep. It is currently marketed for sheep in Europe, New Zealand, and Australia, but it remains unknown whether this class will be investigated for equine use.

Adverse reactions to anthelmintic therapy

Despite the fact that prevention of clinical disease remains one of the traditional motivations for deworming horses, anthelmintic treatment occasionally triggers specific parasitic problems, even if administered according to label directions. Some of these parasitic syndromes are associated with guarded to poor prognoses, so it is important to recognize these risks and to take appropriate precautions to minimize the threat.

Ascarid impaction of the small intestine

Although ascarid impactions can occur spontaneously, they are most often triggered by prior deworming with an anthelmintic that has a paralytic mode of action (Schusser *et al.* 1988; Cribb *et al.* 2006). Affected horses are usually less than 1 year of age. Small intestinal impactions are generally associated with a guarded to poor prognosis for survival, and the condition may require surgical intervention. Patients often develop ileus during postoperative recovery, and require intensive care. In complicated cases, intussusceptions and intestinal ruptures can occur and the prognosis worsens. In the literature, ascarid impactions are associated with recent administration of macrocyclic lactones or pyrantel, but a similar association has not been found with BZDs. The differing modes of activity provide a plausible explanation because the onset of activity is slower for BZDs than for the paralytic anthelmintics. Perhaps BZDs should be recommended as a drug of choice for treatment of adult *Parascaris equorum* infections, - particularly when large worm burdens are suspected.

Timing of ascarid treatments can be a complicated matter. On one hand, ascarid larvae return to the gut by about 4 weeks postinfection, but the individual worms continue to increase in size until approximately 3 months of age. The risk of impaction is likely to increase with the size of the worms. Therefore, an initial anthelmintic treatment at weaning carries a greater risk of adverse sequelae than if the first ascarid treatment had been administered during the suckling phase. In contrast, the efficacy of anthelmintic treatment against any nematode generally improves as the individual worm ages. A case in point, the efficacy of oxibendazole (10 mg/kg) against patent (i.e., mature) ascarid infections ranged from 94% (Lyons *et al.* 2008a) to 100% (Drudge *et al.* 1979) when measured by fecal egg count reduction test (FECRT), whereas the same dosage only removed 44.5% of ascarids from the gut when infections were 28 days old (Austin *et al.* 1991). Adulticidal ascarid treatments should not begin until foals are at least 60 days of age, and should be administered at the maximum tolerable intervals to minimize selection for resistance. Of course, the downside of nonsuppressive ascarid control is environmental contamination with an infective stage that can survive for many years.

Larval cyathostominosis

Larval cyathostominosis can also occur spontaneously, but it more often follows an anthelmintic treatment within 2 weeks prior to the onset of clinical signs (Reid *et al.* 1995). Horses at risk are typically less than 5 years old, and have been treated recently with a nonlarvicidal drug during a season when active transmission is minimal (i.e., the "off-season"). In other words, it tends to happen when a majority of the cyathostomin burden is comprised of encysted larvae. In a cold, temperate climate, most cases of larval cyathostominosis occur during late autumn, winter, and early spring, whereas the onset tends to occur during summer and early autumn in warmer climates. Considering that virtually all horses are infected with cyathostomins, this complication is a rare event. Horses that develop the syndrome may have a history of being treated inadequately during the preceding grazing season, or exposure to over-stocked and highly infective pastures. These circumstances allowed them to accumulate large burdens of encysted larvae.

Deworming young horses for strongyles during the off-season should be performed with care, and one might give serious consideration to larvicidal anthelmintic regimens at that time. Although one of the authors (MKN) has observed larval cyathostominosis following treatment with a larvicidal anthelmintic, the risk appears to be considerably lower. Two choices currently exist for cyathostomin larvicidal therapy: (1) a 5-day course of FBZ (10 mg/kg), and (2) a single dose of moxidectin (400 µg/kg). Both options have exhibited good efficacies against encysted burdens, but considering the prevalence of BZD resistance in most managed horse populations, the FBZ regimen often may not work as expected. In addition, studies have shown that the inflammatory reaction in the mucosal membranes is minimal after moxidectin treatment (Reinemeyer 2003, Steinbach *et al.* 2006), while one study found it to be substantial following FBZ (Steinbach *et al.* 2006). In consideration of these factors, moxidectin should be the drug of choice for horses at risk of developing larval cyathostominosis. Similarly, moxidectin remains the drug of choice for treating a horse with a severe clinical condition caused by cyathostomins.

Post-dosing colics

An effective anthelmintic program clearly reduces the prevalence of colic in a herd over time (Uhlinger 1990; Hillyer *et al.* 2002), but an elevated risk of colic during the initial, post-treatment period has been reported (Kaneene *et al.* 1997; Cohen *et al.* 1999; Barrett *et al.* 2005). These colics could have been attributed to mechanical obstruction if ascarids were present. Alternatively, it has been hypothesized that mucosal reactions to the expulsion of dead worms or the release of chemical or immunologic mediators

might affect intestinal motility or the alimentary blood supply (Love 1992).

Anaphylactic reactions

Although it is intuitively feasible that the death of parasites within tissues could result in anaphylaxis, the treatment of migrating strongyles and ascarids, as well as encysted cyathostomins, has not been associated with adverse events of this type. In fact, studies evaluating the effects of ivermectin and moxidectin have found good healing of parasitic lesions with only limited inflammatory reaction to the dead parasites (Reinemeyer 2003; Slocombe *et al.* 1987, Steinbach *et al.* 2006). The rare examples of anaphylactic reactions following anthelmintic treatment have been associated with treatment of filarial parasites, particularly *Onchocerca* spp. (Wildenburg *et al.* 1994; Mancebo *et al.* 1997) and *Dirofilaria immitis* infection in dogs (Boreham & Atwell 1983). Horses seem to experience milder inflammatory reactions following treatment with moxidectin compared to ivermectin (Monahan *et al.* 1995; Mancebo *et al.* 1997).

Anthelmintic treatment regimens

A variety of treatment programs have been described over the years, but most were based on concepts of the interval-dose program, which was introduced in the 1960s (Drudge & Lyons 1966). Essentially, these treatment regimens attempt to prevent or reduce parasite transmission, but often without adequate consideration of the size or the composition of the parasite burden. With the advent of efficacious, broad-spectrum dewormers in the 1960s, the achievement and perpetual maintenance of parasite-free premises was considered a realistic goal. The experiences of recent decades, however, have demonstrated that the parasite-free horse farm does not exist, and attempts to impose such a venue on Mother Nature merely create strong selective pressure for the development of anthelmintic resistance. Modern parasite control strategies, therefore, have moved toward acceptance of some level of parasitism, and maintenance of sustainable control objectives. The following section offers a brief description of the main therapeutic principles that have been applied historically to achieve equine parasite control (Love 1993).

Interval-dose program

As mentioned previously, interval dosing involves treatment of all horses on a farm at fixed intervals, all year long. The original, recommended treatment interval was 2 months, but timing should be adjusted to match the expected ERP of the anthelmintic used most recently. Interval dosing is an example of suppressive treatment, in which the objective is to minimize contamination by matching treatment intervals with the ERPs of effective products.

Interval-dose programs usually do not involve parasite surveillance, diagnostics, or evaluation of anthelmintic efficacy. In addition, climatic influences and seasonal differences in parasite transmission are given little, if any, consideration. Rather, rote utilization of broad-spectrum anthelmintics has been viewed as a solid strategy to ensure control of all important parasites.

The principles of the interval-dose program are very simple, and easy to implement. It is a one-size-fits-all, cook-book recipe that does not require expenditures for diagnostic surveillance, or consideration of complications such as age of horses, stocking density, or seasonal differences. As a result, interval dosing has become the common standard in managed horse populations worldwide (Anonymous 1999; Lloyd *et al.* 2000; O'Meara & Mulcahy 2002). However, most equine parasitologists now believe that the interval-dose program and its derivatives have been the main contributors to current levels of anthelmintic resistance in equine establishments (Kaplan & Nielsen 2010).

Strategic dosing

As opposed to interval dosing, strategic dosing takes seasonal differences into consideration. In this program, treatments are primarily administered during the active grazing season, but are usually still performed without diagnostic work. Hence, all horses

receive the same treatments at fixed times during the year. As a result, the treatment intensity can be considerably lowered when compared to the interval-dose approach.

Continuous, daily treatment

This approach involves the daily administration of pyrantel tartrate to selected horses, either perennially or throughout the grazing season. Daily administration of a dewormer should kill ingested L_3 before they invade mucosal tissues, and would have therapeutic activity against recently emerged adults. Consequently, egg counts of horses receiving daily pyrantel tartrate should remain low while receiving the regimen. Due to national differences in drug approvals and marketing strategies, daily pyrantel - tartrate formulations are only available in North America. Pyrantel pamoate/embonate resistance is much more prevalent in North America than anywhere else in the world; so it has been hypothesized that this may comprise cross-resistance which was fostered by the daily use of pyrantel tartrate (Kaplan & Nielsen 2010).

Selective therapy

Selective therapy (or targeted treatment) is markedly different from the control strategies described previously, because it abandons whole-herd treatment and selects only certain individual horses for anthelmintic therapy. Selective therapy reduces treatment intensity by considering the distribution of parasites in mature members of the herd.

As a general rule, parasites are always over-dispersed among their hosts (Galvani 2003) so that a minority of animals harbors a majority of the parasites. It is entirely logical to allocate specific treatments to these "wormy" individuals, while others in the herd are dewormed less frequently, and possibly even left untreated. Implementation of this approach requires systematic surveillance with tools that are capable of categorizing infection intensity. A number of diagnostic methods have been employed to identify and segregate hosts with differing susceptibilities to parasitism. In ruminants, for example, this principle has been implemented by classifying gradations of mucosal pallor as an indication of anemia caused by the highly pathogenic trichostrongylid, *Haemonchus contortus*. This system, known as FAMACHA® (Malan *et al*. 2001), has proven highly useful for small ruminants in areas where *Haemonchus contortus* predominates. Other proposed methods of selecting individuals for treatment are based on body-condition scoring or regular body weights, and treating only those with weight loss or suboptimal weight gain (reviewed by van Wyk *et al*. 2006).

Recommendations for selective therapy of horses are based on quantitative fecal analysis of all equids on the premises, and treatment of only those with counts exceeding a predetermined cutoff value (Duncan & Love 1991; Gomez & Georgi 1991). The selection of threshold values for treatment has been a contentious issue because one standard value might not be equally valid for different parasites, horses, or management systems. Until recently, the choice of cutoff values has not been evidence-based because no equine studies had investigated the relationship between egg counts and worm counts. One paper examined designated cutoff values by a number of parasitology laboratories, and found a weak consensus for anthelmintic treatment when egg counts reached approximately 200 EPG (Uhlinger 1993). Using historical egg count and worm count data generated over 50 years of research at the University of Kentucky, it was recently reported that no direct, linear relationship could be established between egg counts and worm burdens. However, it was determined that horses with strongylid egg counts below 500 EPG harbored significantly smaller worm burdens than those with counts exceeding 500 EPG (Nielsen *et al*. 2010). These findings support the establishment of a threshold value in the 100–500 EPG range. However, many of the horses in the Kentucky data set were less than 2 years of age, and selective therapy is currently being promoted only for mature horses. Evaluation of a data set of fecal egg counts from horse herds in the southeastern U.S. (Kaplan *et al*. 2004) revealed that only one half of the horses represented would have been treated if a hypothetical cutoff value of 200 EPG had been implemented. Still, using a drug with a 99% efficacy in these populations would have reduced the overall strongyle egg contamination by 96% (Kaplan & Nielsen 2010).

Concerns have been raised regarding the risk of clinical disease when applying selective therapy in horses. The consequences of under-treatment have not yet been evaluated, but we are reminded that cyathostomins are generally not very pathogenic, and clinical disease is the exception rather than the rule.

In Denmark, anthelmintic use is legally restricted to therapeutic applications, and rote or preventive treatments are not

permitted. Selective therapy is used widely for Danish horses, and recent studies have determined that *Strongylus vulgaris* is exhibiting resurgence in prevalence on many farms, mostly in asymptomatic horses (Bracken *et al*. 2012). The strict disallowance of prophylactic treatments has resulted in a significant share of horses not receiving any treatments if they consistently have low egg counts. Based on this extreme example, perhaps selective therapy recommendations should include one or two treatments of all horses during the annual cycle, with the main objective of interrupting large strongyle life cycles. Additional treatments would then be based on individual fecal egg counts.

Anthelmintic resistance

As described previously in this chapter, anthelmintic resistance has become so prevalent in equine parasites that resistance is likely present in all managed horse herds worldwide (see Table 7.1). This circumstance should not surprise us because resistance is a biological consequence of anthelmintic treatment, and serves as an excellent, contemporary example of Darwinian selection. We cannot reverse or avoid resistance, but we can affect the rate at which it is propagated. To do so, we must understand some of the basic mechanisms by which resistance develops.

An important characteristic of helminth parasites is their incredibly broad genetic diversity. A very wide selection of genes exists within any worm species, and some of those genes can encode mechanisms that are manifested as anthelmintic resistance. As a fellow parasitologist is fond of saying, "Somewhere in the world, worms exist that are resistant to a class of drugs that hasn't been discovered yet." In addition to these background genes, spontaneous mutations can occur in a small number of individuals within the population.

Table 7.1 Current levels of resistance by major nematode parasites to three anthelmintic classes in managed horse herds.

Drug class	Cyathostomins	Large strongyles	*P. equorum*
Benzimidazoles	Widespread	None	None
Pyrimidines	Common	None	Early indications
Macrolide lactones	Early indications	None	Widespread

Regardless of origin, the frequency of resistance genes is not likely to increase unless that genetic combination somehow confers an advantage over the rest of the parasite population. Genetic change comes about as a result of factors that are termed selection pressures, and for anthelmintic resistance, the selective advantage derives from anthelmintic treatment. Whenever a deworming treatment is administered, the ≥99.9% of the population that is susceptible to the drug is removed, and cannot resume dissemination of its genes until the ERP expires. Because resistant worms are not killed by the treatment, however, they can continue to reproduce throughout the ERP, in the total absence of the usual competition. In this fashion, the resistant worms are able to contribute more offspring to the population than the average member, and the genotypic frequency of the resistance factors increases incrementally within the population as a whole.

Various management factors can accelerate or intensify selection pressure for the development of anthelmintic resistance. These include exclusive use of a single class of anthelmintic, which allows resistant worms to reproduce continuously. In contrast, susceptible worms can only lay eggs during the interval between expiration of the ERP and the next scheduled treatment. Another factor is excessively frequent use of a drug class. This practice minimizes *refugia* because susceptible worms are denied an opportunity to reproduce.

A number of genes have been identified and associated with anthelmintic resistance to the various drug classes. But, resistance is widely believed to be multigenic, so the few genes identified to date may just be the tip of the iceberg. Hence, anthelmintic resistance is not just a matter of a single-point mutation in the genome, but rather a combination of different genetic determinants. In BZD resistance, for example, specific mutations have been identified in the gene encoding beta-tubulin (Beech *et al*. 2011). The

genetic mechanisms are less well understood for other nematocidal drugs. Ivermectin resistance has been associated with changes in genes encoding GluCl, but this precise mutation does not appear to be very important outside of the laboratory (Beech *et al.* 2011). Recently, increased expression of P-glycoprotein (Pgp) multidrug transporters has been implicated as having a role in resistance. Interestingly, this mechanism does not appear to be linked with any particular drug class, so cross-resistance among various drug classes might occur (Beech *et al.* 2011).

Anthelmintic resistance in helminth parasites differs in a number of ways from antibiotic resistance in bacteria: (1) Parasites are eukaryotic, and their genes are organized in chromosomes in a manner similar to vertebrates. (2) Reproduction is sexual, and requires exchange of genetic materials between males and females. (3) Generation intervals are comparably much longer, with only one to a few parasite generations per grazing season. (4) Parasite life cycles involve stages that develop in the environment, where they are exposed minimally to anthelmintics. (5) Similarly, life cycle stages within the host may not be exposed to selection pressures when they occupy sequestered, privileged locations. A pertinent example is found in encysted cyathostomins, which are only exposed to those few drugs with demonstrated larvicidal activity.

The consequence of the last three factors is that only a portion of the parasite population is exposed to an anthelmintic at the time of any treatment. But, for resistance to achieve clinical significance, the alleles encoding resistance must accumulate through several successive generations. Accordingly, drug resistance develops very slowly in helminths. Apropos to this discussion, ivermectin- and moxidectin-resistant populations of *P. equorum* were not reported until after nearly 20 years of intensive use of these products (Boersema *et al.* 2002; Hearn & Peregrine 2003).

Within a nematode species, the various life cycle stages often exhibit different susceptibilities to anthelmintics. In general, juvenile and larval stages of strongyles and ascarids are less susceptible than adults, so treatment of a prepatent population may not be as effective as treatment after patency. Preadult nematodes are usually the dose-limiting stages for anthelmintic treatment, and resistance is likely to be detected first in juvenile worms. Indeed, the earliest indication of ivermectin and moxidectin resistance in cyathostomins has been the unprecedented survival of L_4 in the lumen of the gut after treatment (Lyons *et al.* 2009, 2010). A similar circumstance is suspected for *P. equorum*, and it does not augur well that L_4 cyathostomins routinely survive pyrantel treatment.

It is crucial to recognize that anthelmintic resistance is a permanent, genetic feature of a nematode population. Despite total avoidance of a particular drug class, a parasite population can remain resistant for decades. As a case in point, thiabendazole resistance was induced in a horse band at the University of Kentucky during the 1970s, and the affected premises have been occupied by a closed herd for the past 40 years. Yet, BZD resistance was still present at this site after 22 years of rigorous abstention from BZD drugs (Lyons *et al.* 2007).

Parasite *refugia*

Refugia is a term that refers to any portion of a population that is not exposed to a selection pressure for genetic change. A *refugia* functions as a reservoir of susceptible genes. In relation to anthelmintic resistance, parasites in *refugia* are those stages of the life cycle that are not exposed to a drug at the time of deworming. Logically, only parasitic stages within the horse are exposed to an anthelmintic during treatment, so all stages in the environment (egg, L_1, L_2, L_3) are considered to be in *refugia*. Although they are present within the host, encysted cyathostomin larvae are also classified as being *in refugia* whenever nonlarvicidal anthelmintics are administered. A very significant portion of *refugia* is all of the parasites within any horse that is left untreated (e.g., in the implementation of a selective therapy program).

Refugia has become the central tenet in understanding the development of anthelmintic resistance in parasites (van Wyk 2001). Because it serves as a reservoir of susceptible genes, maintaining the largest possible *refugia* should minimize selection for anthelmintic resistance. Let us consider this point from another perspective. The only pattern of anthelmintic use that could never result in resistance is complete abstention from deworming. If drugs were never used, then none of the parasite population is exposed, so *refugia* is maximal. Whenever a large portion of a worm population is exposed frequently and repeatedly to anthelmintics, the selection pressure for development of resistance is very high. This description fits interval dosing quite succinctly, especially when larvicidal products are used. If, on the other hand, only 20% of the parasite population is exposed to a drug, the selection for resistant parasites will be diluted by the 80% *in refugia*. This circumstance reflects what selective treatment

approaches attempt to accomplish. Again, a large *refugia* predicts a slower development of resistance.

Now that we understand some basic concepts of parasite *refugia*, we can appreciate another difference between antibiotic resistance in bacteria and anthelmintic resistance in worms. Bacterial populations are rarely *in refugia*.

From Chapter 3, we learned that environmental and climatic factors greatly influence parasite development and transmission, and thus we know that some seasons have larger parasite *refugia* than others (Nielsen *et al.* 2007). Although L_3 may survive under the snow cover during winter, the relative numbers of larvae ingested during the following spring are much smaller than the numbers ingested during the grazing season. So, treatment when the *refugia* is small (e.g., winter treatments in cold climates) would theoretically increase the selection pressure for anthelmintic resistance.

The role of parasite *refugia* in the development of resistance is now a widely accepted concept, but little field evidence has been generated to support it. Field studies with sheep have confirmed its existence (Martin *et al.* 1981; Waghorn *et al.* 2008), and computer models have clearly illustrated the importance of unselected populations (Barnes *et al.* 1995). However, no data have been generated in studies with horses to characterize the role of parasite *refugia*.

Drug rotation

Rotation among dewormers has long been viewed as a reliable strategy for avoiding anthelmintic resistance. The underlying theory is that parasites resistant to drug "A" will still be susceptible to anthelmintic "B" if it has a different mode of action. Treatment with "B" would remove adults resistant to "A," so the latter would not gain a sustained reproductive advantage. This all sounds very logical, but there is absolutely no evidence to support it. One equine study clearly showed that rotating drugs with each treatment did not appear to slow the development of resistance (Uhlinger & Kristula 1992). Furthermore, computer modeling with sheep nematodes concluded that rotating drugs does not prevent accumulation of resistant genetic alleles, and therefore does not slow down the development of resistance (Barnes *et al.* 1995). The flaw in the logic is that the resistance alleles are still present in all the environmental stages, so removing adults does not eliminate the trait from the population. Further, it appears likely that drug rotation may select for genes such as the Pgps mentioned earlier in this chapter, which confer resistance across different drug classes.

The resistance problem is further compounded for horses because only three classes of anthelmintics are currently available, and the prevalence of resistance is already high for two of the three. Thus, on many farms, rotation is simply not an option. Many, and perhaps most, horse owners labor under the mistaken notion that the usual complement of anthelmintics is still effective in their herd because they have practiced meticulous rotation in the past. Regardless of the treatment schedule implemented, all anthelmintics employed must be checked routinely to ensure that they still deliver acceptable efficacy. Drug rotation is no substitute for routine testing for anthelmintic resistance on the farm.

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Section III

Diagnosis and Assessment of Parasitologic Information

8 Diagnostic Techniques for Equine Parasitism

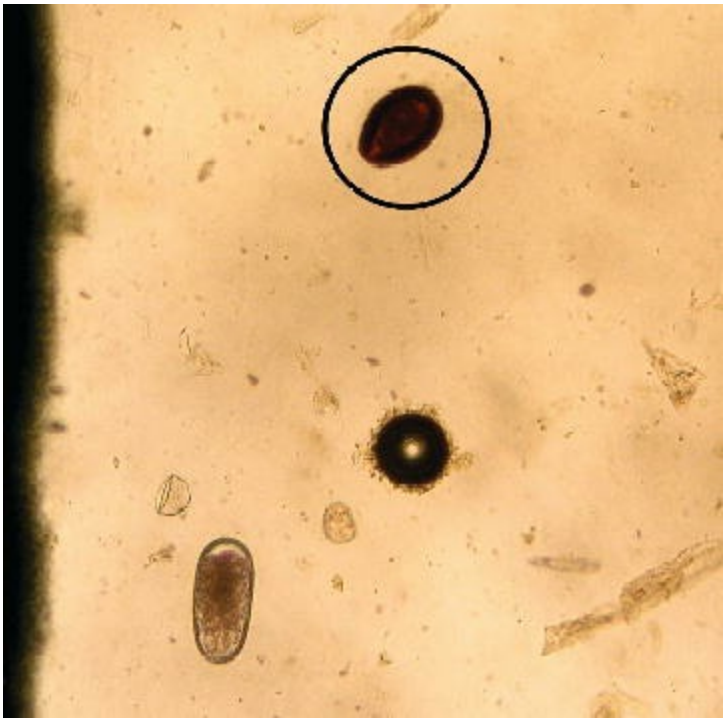
Clinical signs of gastrointestinal parasite infection are nonspecific, and the results of clinical pathology evaluations (e.g., hemogram, serum chemistry) provide only tentative support at best. Parasitic disease is rarely associated with pathognomonic findings, so clinicians are left with pattern recognition. Typical manifestations of parasitism include ill-thrift, a rough haircoat, and pot-bellied appearance. Horses younger than 4 years of age are more likely to be clinically affected by parasites, and clinical parasitism should always be included among the differential diagnoses for this age group. As described in Chapter 2, gastrointestinal helminthiasis can result in colic and diarrhea, but multiple, alternative causes for these signs exist as well.

Certain clinical findings lend strong support to a diagnosis of clinical parasitism. Rectal palpation or ultrasound examination of a thickened and dilated cranial mesenteric artery has been used to diagnose *Strongylus vulgaris* infection in small horses (Greatorex 1977; Wallace *et al.* 1989). Similarly, *Parascaris equorum* are large, echogenic, and easy to recognize, so transabdominal ultrasound has proven useful for diagnosis of ascarid infection in foals and weanlings.

The most useful laboratory parameter is serum protein concentration, which is often reduced in cases of larval cyathostominosis. However, this is not pathognomonic because other gastrointestinal conditions, such as *Lawsonia intracellularis* infection, can cause hypoproteinemia as well. Eosinophilia is classically associated with parasitic migration, but absolute counts are generally inconsistent and difficult to interpret. Therefore, eosinophil counts have no practical value.

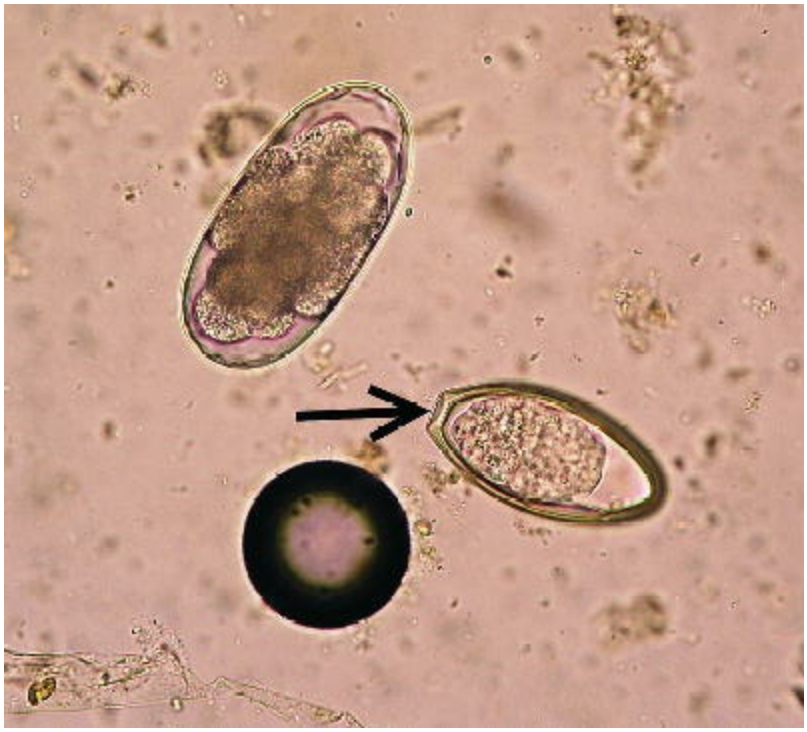
The majority of currently available tools for the diagnosis of parasitism are based on detection or enumeration of parasite progeny (eggs or larvae) shed in the feces from a horse. Positive findings rely on the presence of an adult worm population. This methodology has several practical limitations. First, strongylid parasites are most pathogenic during their larval stages, which are sexually immature and not capable of producing eggs. Second, some parasite species, such as the lungworm (*Dictyocaulus arnfieldi*) or liver fluke (*Fasciola hepatica*), may not reach sexual maturity in the horse, and therefore no offspring are produced. Such prepatent or occult parasite infections are very difficult to diagnose. Despite these shortcomings, fecal examination (coprology) remains the cornerstone of equine parasitologic diagnosis.

Figure 8.1 ***Eimeria leuckarti*** **oocyst (circled). Typical strongyle egg below for size comparison.** (*Source*: Photograph courtesy of Tina Roust and Maria Rhod)



Identification of parasite reproductive products occurring in equine fecal samples is less complicated than for most domestic animals because the eggs are generally the same size. As a rule of thumb, most equine parasite eggs approximate the dimensions of a typical strongyle egg (about 50 µm × 100 µm) and have a smooth surface and identifiable contents. Egg-like objects much larger than this are likely artifacts, such as mite eggs, while much smaller structures are often pollen grains. A few parasites can be identified by the distinct morphology of their eggs. The most unique of these is *Parascaris equorum*, which is described further below. Other distinctive parasite eggs include those of *Strongyloides westeri*, which are most often identified in foals less than 6 months of age. Oocysts of the protozoan parasite *Eimeria leuckarti* are unusually large and have a very distinct appearance (Figure 8.1). These are seen almost exclusively in young horses, and even then only infrequently. Occasionally, eggs of the equine pinworm *Oxyuris equi* may be observed during fecal examination (Figure 8.2), but as a rule, eggs of this parasite are found around the anus and are not passed in the feces. Exceptionally, eggs of the liver flukes *Fasciola hepatica* or *Dicrocoelium lanceolatum* may be identified. Finally, eggs of anoplocephalid tapeworms are easily distinguishable when found on fecal examination, but modifications of diagnostic techniques are required to specifically detect tapeworm eggs (see Section 8.1.7). We present the main coprologic techniques in this chapter.

Figure 8.2 ***Oxyuris equi*** **egg next to a strongyle egg. Note the operculum at one end (arrow).** (*Source*: Photograph courtesy of Tina Roust and Maria Rhod)



Coprology

Fecal flotation

Although parasite eggs may be present in very large numbers, they nevertheless occupy a relatively small volume of any sample of fecal material. Detection of parasite reproductive products in feces requires physical separation of eggs from the bulk of other organic material present, and subsequent concentration in a relatively small volume for convenient microscopic observation. Helminth eggs have a mass (or specific gravity) which is greater than that of water (e.g., H_20 = 1.000), but lower than most other biological materials present in feces. If feces were mixed with tap water, the eggs would sink along with the majority of the fecal

material. If feces were mixed with a dilute solution of some sugar or chemical salt (e.g., sucrose, $NaNO_3$, NaCl, $MgSO_4$, $HgCl_2$) with a specific gravity ≥ 1.18, the helminth eggs would float, but the heavier organic matter would still sink. By transferring such a mixture to a tall cylinder with a relatively small diameter, the floating eggs could be concentrated in a relatively small volume of solution at the very top of the liquid column. This physical isolation of eggs by differences in specific gravity can be expedited by centrifugation. A couple of examples of flotation media recipes are presented in Box 8.1.

Box 8.1 Flotation media

Sheather's sugar solution

Sheather's sugar solution is widely used in North American laboratories. Add 454 g (1 lb) of sucrose (table sugar) to 355 mL of very hot water, or stir into steaming water on a hot plate. Stir until dissolved and allow to cool. Sucrose solution will support the growth of mold at room temperature, so keep refrigerated and use quickly or add 6 mL of formaldehyde (37%) for preservation.

Saturated sugar-salt solution

Saturated salt solution is widely used in European laboratories. Weigh 375 g glucose monohydrate and 250 g sodium chloride. Add distilled water q.s. 1 L total. Warm to 80°C and stir gently until dissolved. A saturated salt solution can be used alone, but by adding the glucose, a specific gravity of 1.25 can be achieved.

Checking specific gravity

The specific gravity of flotation solutions can be measured with a hydrometer. Alternatively, a carefully measured volume of the solution can be weighed with a sensitive laboratory balance. For example, 10 mL of a flotation solution with a specific gravity of 1.25 will weigh 12.5 g.

Fecal flotation is a qualitative technique that can only document the presence or absence of helminth eggs. The lack of standardization in this technique precludes quantitation of egg counts as well as accurate comparisons of egg shedding among horses or in the same horse over time.

Fecal egg counts

Fecal egg counts remain the cornerstone of equine diagnostic parasitology, although recent advances have been made with other methodologies. All egg-counting methods are based on the flotation principle discussed previously. Quantitative techniques differ primarily in that the essential components (feces and flotation solution) are measured carefully, a known representative volume of the mixture is examined, and the numbers of parasite reproductive products are counted and recorded. These elements permit calculation of a diagnostic result (fecal egg count) that is expressed in eggs per gram of feces (EPG). Reproductive products of different species of parasites are counted and reported separately.

Numerous fecal egg-counting methods have been described, such as the McMaster (Gordon & Whitlock 1939), Stoll (Stoll 1923), and Wisconsin (Cox & Todd 1962) techniques. Newer methods such as the FECPAK (Presland *et al.* 2005) and FLOTAC (Cringoli *et al.* 2010) techniques are basically derivations of the McMaster test with higher sensitivities. The various methods differ primarily in how the quantity of feces is measured, and how the eggs are isolated for counting. Some techniques employ passive flotation (Gordon & Whitlock 1939; Presland *et al.* 2005), and other methods require the use of a centrifuge (e.g., Cox & Todd 1962; Roepstorff & Nansen 1998; Cringoli *et al.* 2010) and are inherently more time-consuming.

The most important parameter to consider when deciding which technique to use is the limit of detection of the test. Limit of detection is defined as the lowest, positive fecal egg count (FEC) that can be reported by a certain technique. Most quantitative methods are essentially dilution techniques. One counts the number of eggs in a representative sample of feces, and mathematically extrapolates the number of eggs counted to a standard reporting unit (usually EPG). An egg-counting technique with a limit of detection of 25 EPG, for instance, would not give the same results as a method with a sensitivity of 1 EPG. As an example, if the "true" egg count for a given sample were 17 EPG, the former technique, with a limit of detection of 25 EPG, could yield a result of "0" EPG. This number should not be interpreted verbatim that no eggs were present, but rather that the true number was lower than the limit of detection (i.e., <25 EPG). A test with a sensitivity of 1 EPG, however, could have yielded a

result of 17 EPG for the same sample. Box 8.2 and Box 8.3 give examples of two egg-counting techniques with different sensitivity levels.

The sensitivities of the tests listed previously range from 1–100 EPG. An egg count technique with a high limit of detection (e.g., 100 EPG) is not necessarily inferior to one that is very sensitive. Tests with high limits of detection (i.e., ≥25 EPG) are far less labor-intensive. As an example, if the "true" egg count of a sample were 1050 EPG, and an egg-counting technique with a detection limit of 50 EPG were used, one could determine this result by counting only 21 eggs (21 × 50 = 1050). If a technique with a sensitivity of 1 EPG were used for the same sample, however, one's thumb would have to depress a tally counter 1050 times to achieve the same result. As long as egg count results are roughly "in the same ball park," clinical management decisions would likely be the same regardless of the quantitative technique used. This is true for anthelmintic treatment decisions or the classification of strongyle contaminative potential (SCP). When egg counts are performed as a part of a fecal egg count reduction test (FECRT), however, detection of low FECs post-treatment becomes critical, and more sensitive techniques are preferred. Interpretation of FECRT is discussed further in Chapter 9.

Fecal egg counts can be used for three general purposes: (1) as a diagnostic tool for clinical cases, (2) as a surveillance tool for identifying high egg shedders, and (3) to conduct FECRT. It is important to recognize that FEC results are not equally useful for these three purposes. The following discussion will address these applications individually.

Box 8.2 A simple McMaster egg-count technique

This is a simple McMaster method for routine application in veterinary practice. This technique does not require centrifugation and has a limit of detection of 50 EPG.

Materials

Disposable cups, wooden tongue depressor, disposable plastic pipettes, laboratory balance (scale) with 0.1 g accuracy, cheese cloth (17 thread), McMaster counting chamber, flotation solution with a specific gravity in the range of 10.18–10.25 ($ZnSO_4$, saturated salt, saturated sugar-salt, or Sheather's sugar), compound microscope.

- Weigh out 4.0 g of feces in one of the cups
- Add 56 mL of flotation solution (a useful mnemonic device for the recipe is 4-5-6).
- Mix well and homogenize the suspension with a tongue depressor.
- Pour the suspension through two layers of cheese cloth into another cup.
- Sample the liquid strained through cheesecloth. While stirring vigorously, aspirate a sample of the mixture with a pipette. Holding the pipette nearly horizontal, transfer the strained liquid into one of the chambers of the McMaster slide. Be sure to fill the entire area under the grid, while avoiding bubbles in the chamber. Repeat the procedure and fill the other chamber.
- Wait at least 30 seconds and examine, using the 10× objective of the microscope. Count the total number of eggs within each grid, and add the total number of eggs observed in both counting chambers. Report eggs of different parasites (e.g., strongyles, ascarids) separately.
- Multiply the total number of each type of egg by 50. This is the fecal egg count, reported as eggs per gram (EPG). The limit of detection of this method, therefore, is 50 EPG.

FEC as a clinical diagnostic tool

Overall, the clinical implications of FEC results should be interpreted with great caution, firstly because strongyle infections are ubiquitous but generally not very pathogenic. Therefore, the mere presence of strongyle eggs in the feces has little diagnostic value. Secondly, it should be remembered that prepatent larval phases are generally more pathogenic than adult worms, so a zero egg count does not rule out parasitic disease. Lastly, egg counts have no direct correlation with the size of the worm burden, as demonstrated in a recent retrospective study (Nielsen *et al.* 2010b). The same study illustrated that FECs have a high positive predictive value (PPV) but a low negative predictive value (NPV). In other words, a positive fecal exam accurately indicates the presence of adult worms, but a zero FEC does not guarantee the absence of mature worm populations.

Box 8.3 Modified Wisconsin sugar flotation method

The modified Wisconsin method is an example of a very sensitive egg-counting technique, with a limit of detection of 1 egg per gram (EPG). As opposed to the McMaster technique, this method requires a centrifuge.

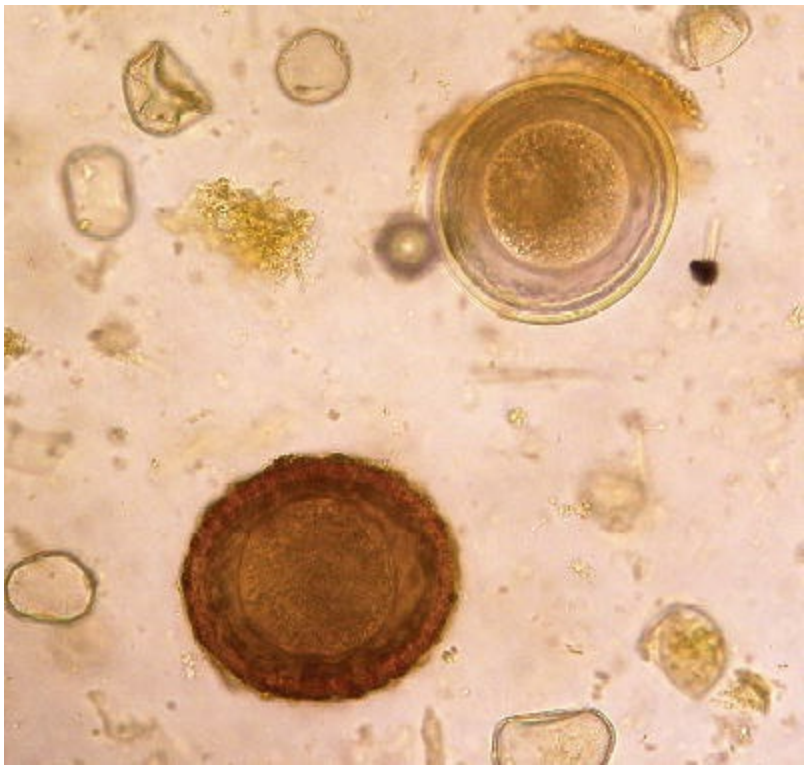
Materials

Disposable cups, wooden tongue depressor, laboratory balance with 0.1 gram accuracy, cheese cloth (17 thread), funnel, Sheather's solution, microscope slides, cover slips (18 × 18 mm), centrifuge, microscope.

- Use a 15 mL test tube to measure 10 mL of Sheather's sugar solution.
- Weigh 1 g of feces and place into a disposable cup.
- Pour the Sheather's solution from the test tube into the cup and mix well.
- Place the funnel into the test tube, and place two layers of cheese cloth inside it. Pour the fecal-sugar solution mixture through the strainer into the test tube. Using the tongue depressor, squeeze the liquid out of any feces retained in the strainer. If one uses disposable paper cups, the second (post-sieving) cup can be pinched to form a narrow spout for pouring the liquid into the test tube.
- Centrifuge the tube for 10 min at 1000 g.
- Fill the tube until a very slight meniscus forms at the top. Place a cover slip onto the meniscus. Avoid over-filling because excess solution will run down the sides of the centrifuge tube, taking eggs with it. Center the cover slip over the test tube.
- Allow to sit for about 5 min, then remove the cover slip and place on a slide labeled with the horse's I.D.
- One can also fill the entire tube with flotation solution and centrifuge with the cover slips in place. But, this requires a centrifuge with a swing-bucket rotor to prevent loss of cover slips during centrifugation.
- Examine the entire cover slip and count the number of eggs observed. The number of eggs counted is the number per gram of feces (EPG). The detection limit of this method is therefore 1 EPG.

FECs may have complex implications in foals and weanlings because they can harbor *Parascaris equorum* as well as strongyles. A FEC will readily yield this information, as both types of eggs are very easy to identify (Figure 8.3). Because some populations of ascarids may be resistant to macrocyclic lactones, and their companion strongyles could be resistant to benzimidazoles and pyrantel, the choice of anthelmintic may depend on whether strongyle or ascarid eggs are more dominant.

Figure 8.3 Two eggs of *Parascaris equorum*. The lighter egg has shed its proteinaceous capsule, a common finding in fecal samples. Several pollen grains can be seen in the picture. (*Source*: Photograph courtesy of Tina Roust and Maria Rhod)



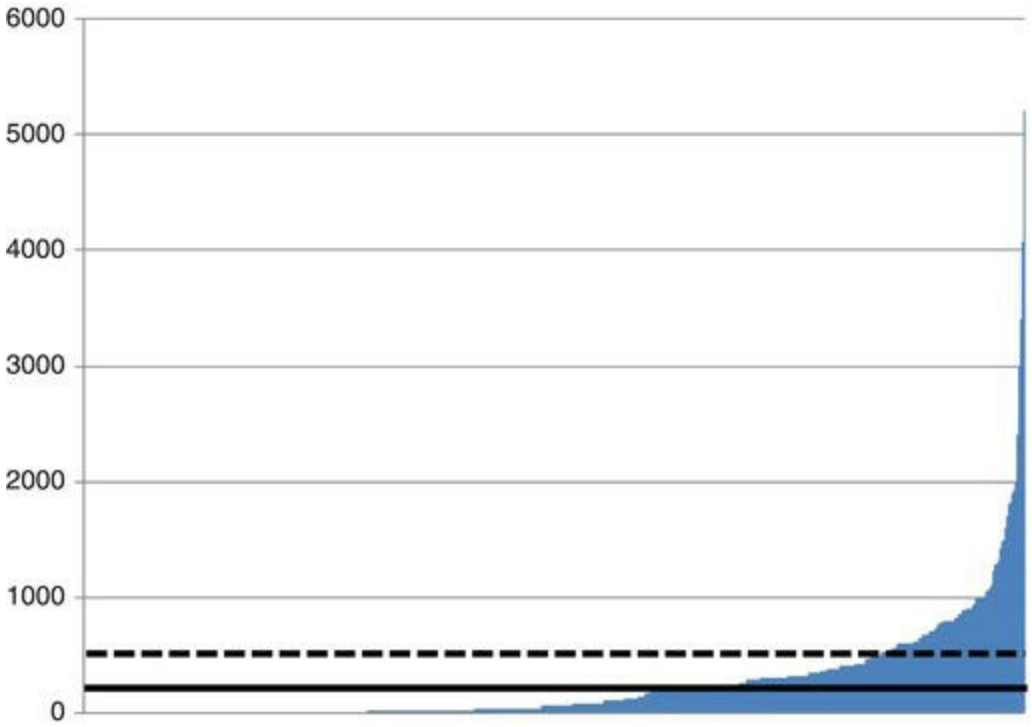
As an overview, FECs have limited value as a clinical diagnostic tool, and treatments should be based most often on the clinical presentation, history, and diagnostic findings other than the FEC.

FEC as a surveillance tool

When FECs are used as a means of parasite surveillance, the main purpose is not to detect adult worms in the horse or to estimate their numbers, but rather to characterize the level of environmental contamination originating from that animal. This parameter has been termed the strongyle contaminative potential (SCP).

Several studies have shown that adult horses tend to maintain roughly similar levels of egg output over time (Nielsen *et al.* 2006; Becher *et al.* 2010). This phenomenon appears to be particularly pronounced in horses with a low SCP, that is, those that consistently shed relatively few eggs. Horses with zero or very low egg counts often maintain this level of egg shedding throughout their adult lives, even in the absence of anthelmintic treatment. Low shedders comprise 40–60% of most adult horse populations. A smaller proportion (often 20–30%) of adult horses in a herd shed moderate numbers of eggs, as depicted in Figure 8.4. A minor portion of the herd (10–30%) can be classified as high shedders, and they are responsible for more environmental contamination with worm eggs than the other two categories combined. In fact, it has been estimated that 80% of all the strongyle eggs passed by a herd originate from only 20% of herd members (Kaplan & Nielsen 2010).

Figure 8.4 Typical distribution of strongyle egg counts in horses. Data from 1566 adult horses on 64 different premises in Denmark. Approximately 60% of horses were shedding < 200 EPG (bold horizontal line), but these only contributed 11% of the total numbers of eggs produced. Another 24% of horses were shedding between 200 and 500 EPG (between dashed and bold lines), corresponding to ~ 25% of the eggs passed. The remainder of horses (only ~ 15%) had egg counts > 500 EPG, but they contributed 64% of the total eggs shed



The consistency of the magnitude of egg shedding is illustrated with farm records in Table 8.1. In this data set, 424 horses were sampled in the spring and fall of three consecutive years. Horses received anthelmintic treatments only when their FEC was 200 EPG or above. The table clearly illustrates the strong tendency of egg counts from horses with low SCP to remain low over time. This phenomenon provides the basis for selective therapy regimens in which horses with the lowest levels of egg shedding are left untreated. It is important to emphasize that this approach can only be applied to adult horses. The principle of selective therapy is described further in Chapter 9.

Egg counts have considerable variability, and one should not expect identical results for repeated egg counts, even if using multiple samples from the same fecal ball. As a rule of thumb, the variability of any given egg count is ~50% (Uhlinger 1993). In other words, a FEC result of 1000 EPG represents a potential range of 500–1500 EPG. Thus, a quantitative result for a horse with a "true" moderate egg count might occasionally fall into the low range. Thus, that animal would remain untreated if the decision were based on a single egg count. However, from a herd perspective, the overall reduction of egg output would not be affected significantly if a few horses with marginal egg counts were misclassified as low or moderate shedders. If more accurate results are warranted, however, egg counts from the same sample can be repeated, and a mean value calculated.

Table 8.1 Consistency of fecal egg count levels in individual horses over time.

Source: Reprinted from *Veterinary Parasitology*, 35/3–4, Nielsen, M.K., Haaning, N., Olsen, S.N., Strongyle egg shedding consistency in horses on farms using selective therapy in Denmark, 333–335, Copyright (2006), with permission from Elsevier.

Result of two previous egg counts	Result of third egg count	Probability (%)
0, 0	0	82
0, 0	< 200	91
< 200, < 200	< 200	84
≥ 200, ≥ 200	≥ 200	59

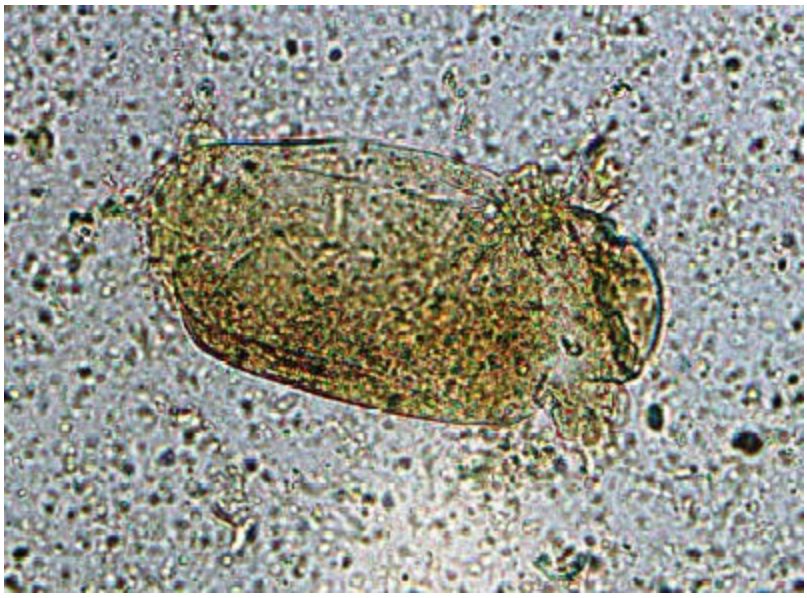
In this study, 424 horses were analyzed twice yearly, in spring and autumn, for 3 consecutive years (Nielsen *et al*. 2006). Horses received anthelmintic treatments if their egg count were 200 EPG or higher. The table predicts the outcome of a subsequent fecal egg count, when the results of two previous sampling occasions were known.

Routine laboratory screening can be applied to classify horses as low, moderate, or high strongyle egg shedders. Low shedders generally have egg counts below the predetermined cutoff value for treatment (usually in the 100–500 EPG range), and high

shedders typically exhibit more than 1000 EPG. Restricting treatment to horses with higher egg counts effectively provides overall reduction of egg shedding by the herd, and simultaneously diminishes the selection pressure for anthelmintic resistance (see Chapter 7 for a discussion of selective therapy).

Early studies in Great Britain demonstrated that strongyle egg shedding also exhibits seasonal variations (Poynter 1954; Duncan 1974). In a northern temperate climate, egg counts were markedly lower during winter, even in the absence of anthelmintic treatment. Over the spring months, a gradual increase was observed from late winter to early summer. This pattern is another example of how parasites spend their resources wisely. Because environmental conditions are unfavorable for egg hatching and larval development during winter months, it is not a good use of limited resources to pass high numbers of eggs. It is possible that a similar pattern can be observed during summer months in warmer climates. Altogether, this suggests that FECs are more reliable during the months of the active grazing season, and it is recommended that they are mainly performed during this time.

Figure 8.5 Ciliate cysts occur normally in equine fecal samples, and are similar in size to strongyle eggs but have an irregular outline. (*Source*: Photograph courtesy of Tina Roust and Maria Rhod)



For reliable results, fecal samples should be collected and stored appropriately to reduce variability. Fecal samples should be collected when fresh, if possible. However, studies have shown that samples collected from the stall floor are reliable for the first 12 h after defecation, if egg counts are performed immediately without further storage (Nielsen *et al.* 2010a). Airtight storage appears to be critical as egg hatching is an aerobic process, and the minimal availability of oxygen in an airtight container will help to preserve the eggs for flotation. Samples kept in airtight plastic bags at room temperature were reliable for the first 24 h, but counts declined significantly thereafter. Refrigeration will maintain reliable samples for at least 5 days, and perhaps considerably longer (Nielsen *et al.* 2010a). Freezing fecal samples reduced egg counts by 20–30% during the initial 24 h, but no further egg loss was observed during an additional 4 days. Egg counts quickly declined during storage at 38°C (100°F) (Nielsen *et al.* 2010a).

Various artifacts in fecal samples are sometimes misidentified as parasite eggs. These "pseudoparasites" include pollen grains, mite eggs, and ciliate protozoa (Figure 8.5).

Detecting anthelmintic resistance

Using fecal egg counts to detect anthelmintic resistance is addressed in Chapter 9 (see Section 9.1).

Fecal egg count summary

Fecal egg counts are mediocre to poor clinical diagnostic tools. They reflect the presence of adult parasite populations, but not pathogenic larval stages. Negative predictive value is modest, implying a risk of false-negative results. Above 500 EPG, the

absolute magnitude of an egg count is not correlated to the size of the adult strongyle worm burden. Despite these limitations, routine FECs are recommended on every horse farm for two purposes: evaluation of anthelmintic efficacy and identification of low, moderate, and high egg shedders for a tailored control strategy.

It is recommended that laboratories performing fecal egg counts routinely validate their techniques against established laboratories.

Baermann Technique

The Baermann technique is used to recover live parasite larvae from a fecal sample. Typically, the sample is placed in lukewarm tap water for 24 h. In an aqueous medium, larvae will swim out of the feces and into the water. Because many larvae tend to be positively geotropic (i.e., swim toward gravity), they congregate in the bottom of a container, wherefrom they can be collected and examined. The Baermann technique has three potential applications in equine parasitology.

(1) Detection of first-stage larvae (L_1) of *D. arnfieldi* (lungworm). But, as described in Chapter 1, lungworms are primarily found in donkeys and rarely reach sexual maturity in horses. Therefore, a Baermann procedure is not very useful for horses, but can be applied to donkeys.

(2) Recovery of cyathostomin larvae newly emerged from the mucosal membranes in horses with suspected larval cyathostominosis (Olsen *et al.* 2003). This technique is applicable only to very fresh specimens, and it requires some attention to distinguish between the L_4/L_5 emerging from cysts and L_1 which hatched during sedimentation at room temperature. See Figure 8.6. This method has not yet been thoroughly validated, but in one author's experience (MKN), false-negatives can occur in cases of larval cyathostominosis.

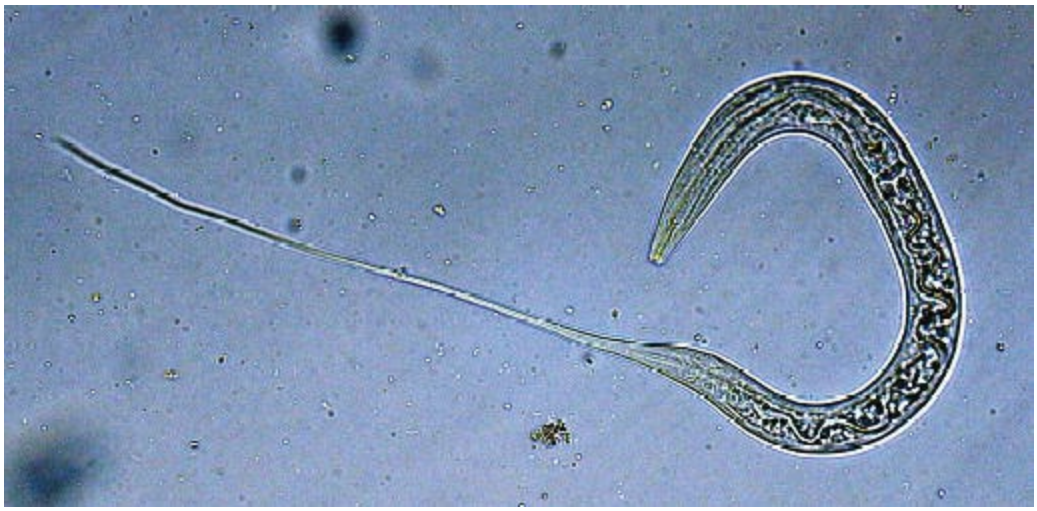
(3) Harvest of L_3 after coproculture. See following text.

Larval cultures

Strongyle eggs are very similar in morphology and generally cannot be identified to the genus or species level. Although subtle differences have been reported among L_1 and L_2 (Ogbourne 1971), diagnoses are never based on the examination of these stages. L_3, however, have more distinct morphologic characteristics which allow several strongyle species or genera to be identified (Russell 1948; Bevilaqua *et al.* 1993). Culturing of feces (coproculture) and subsequent identification of L_3 have practical applications both in research and in practice.

All coproculture techniques essentially incubate feces near room temperature (65–78°F) for periods of up to 2 weeks. Larvae which hatched from eggs in this medium and developed to L_3 are harvested by the Baermann technique. Feces can be mixed with an inert material like vermiculite or granular charcoal to delay evaporation and to facilitate oxygenation, but the consistency of equine feces makes it an excellent culture medium even without additives. During the culture period, samples should be monitored daily for desiccation and supplemental water added if needed. Exposure to direct sunlight should be avoided.

Figure 8.6 First-stage strongyle larva (L1) harvested from a Baermann apparatus. Note the characteristic tail



All strongylid larval stages (L_1, L_2, L_3) exhibit a characteristic whiplash tail (Figures 8.6 to 8.9), but only the L_3 have a double-layered cuticle (larval sheath) and distinct intestinal cells. *Strongylus vulgaris* larvae are the most readily distinguishable because they possess a high number (28–32) of distinct intestinal cells (Figure 8.7), and are often 50–100% larger than larvae of other species. No other strongyle larvae exhibit more than 20 cells, but several have 16–20 intestinal cells. Consequently, *S. edentatus* (Figure 8.8) must be distinguished from *Triodontophorus* spp. (Strongylinae), *S. equinus* from *Poteriostomum* spp. (Cyathostominae), and *Oesophagodontus robustus* (Strongylinae) from *Trichostrongylus axei* (Trichostrongylidae) by means of intestinal cell shape and other morphologic characteristics (Russell 1948; Bevilaqua *et al.* 1993). With the exceptions of *Poteriostomum* spp. (16 cells) and *Gyalocephalus capitatus* (12 cells), cyathostomin species all have eight intestinal cells and are easily distinguished from *Strongylus* species, but not from each other. Although some investigators have been able to subdivide eight-cell cyathostomin larvae into subgroups based on morphometric characteristics (Kornas *et al.* 2009, Bevilaqua *et al.* 1993), the most important practical reason for performing larval cultures is to detect the presence of *S. vulgaris*.

To screen routinely for *S. vulgaris*, practitioners might consider pooling samples from several horses into one culture. However, *S. vulgaris* contributes a very small percentage of the strongyle eggs shed on most farms, and infections can easily be overlooked when samples are pooled (Bracken *et al.* 2012). Any larval culture will be dominated by cyathostomin larvae, which are easily recognized (Figure 8.9).

Figure 8.7 Third-stage larva (L3) of *Strongylus vulgaris*. The larva can be identified to species level based on the presence of 28–32 distinct intestinal cells. (*Source*: Reprinted from *The Veterinary Journal*, 174/1, Nielsen, M.K., Kaplan, R.M., Thamsborg, S.M., Monrad, J., Olsen, S.N., Climatic influences on development and survival of free-living stages of equine strongyles: Implications for worm control strategies and managing anthelmintic resistance, 23–32, Copyright (2007), with permission from Elsevier)

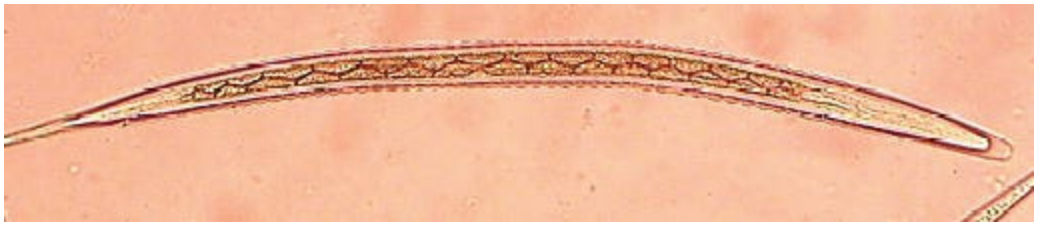


Figure 8.8 The intestinal cells of *Strongylus edentatus* are slender, elongated, and ill-defined, which makes it somewhat challenging to obtain an accurate count. *Triodontophorus* spp. larvae also have 18–20 intestinal cells, but they are well defined and more rectangular

A recent study used historical data to validate larval culturing for diagnosis of infection with two species of large strongyles (Nielsen *et al*. 2010b). Coproculture foretold the presence of *S. vulgaris* and *S. edentatus* adults at necropsy with a high PPV. Larval culture yielded occasional false-negative results for both species, however, and had medium NPVs. No linear correlation was observed between counts of larvae per gram (LPG) of feces and adult worm burdens.

Lugol's iodine solution is routinely used to kill larvae and to improve the visibility of intestinal cells before examining the sample microscopically. Lugol's iodine halts the motility of larvae, but will make intestinal cells indistinguishable after about 5 min. Chilling larvae by holding microscope slides on a cold thermal block also diminishes motility, but larvae are quickly reactivated by heat from the microscope lamp.

Perhaps the most common complication of larval culture is contamination by free-living nematodes. Because free-living nematodes may complete an entire life cycle within culture medium, they can rapidly overwhelm the parasitic larvae present. Samples collected from the ground or stall floor are likely to be contaminated with free-living nematodes, even if collected fresh. Free-living nematodes are easy to distinguish from strongyle larvae; they lack the typical sheath and whiplash tail, have no distinct intestinal cells, and many sizes and developmental stages, including adults, are present (Figure 8.10). In addition, the morphology of the esophagus of free-living nematodes is very characteristic (Figure 8.11).

Figure 8.9 Third-stage cyathostomin larva (L3). Note the eight intestinal cells arranged in one row, and the double-layered cuticle characteristic of this stage (arrows). (*Source*: Photograph courtesy of Tina Roust and Maria Rhod)

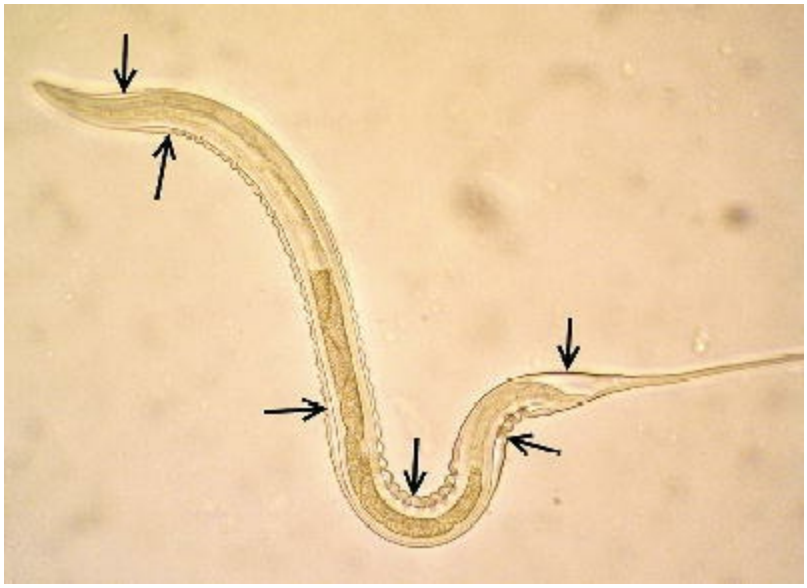
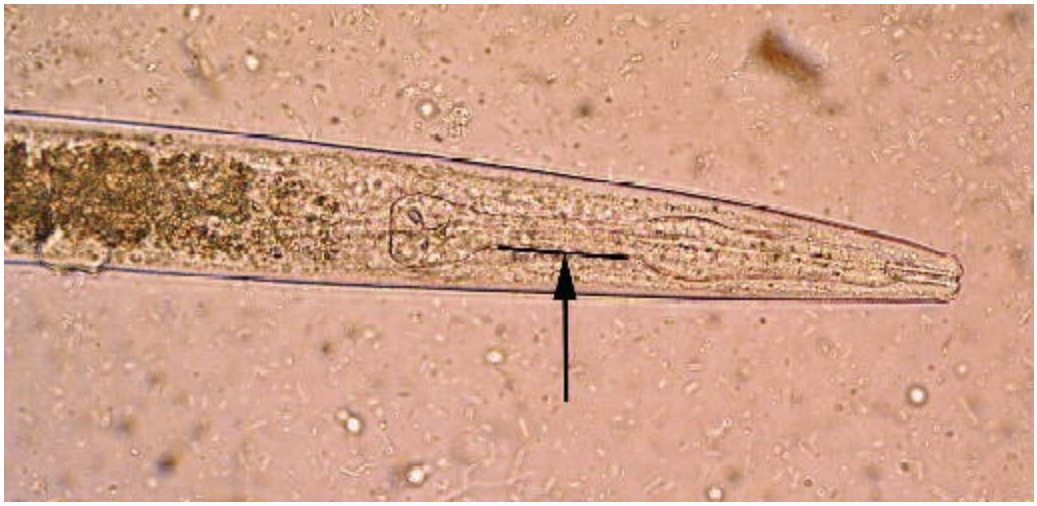


Figure 8.10 Contamination of a larval culture with free-living nematodes. Note the presence of different developmental stages and sizes, as well as the absence of characteristic, long, strongyle tails

Figure 8.11 Free-living nematodes can be recognized by the shape of the esophagus, which features two bulbs separated by a narrow isthmus (arrow). (*Source*: Photograph courtesy of Tina Roust and Maria Rhod)



Although larval cultures are technically simple to perform, it calls for some vigilance, is time-consuming, and requires 2 weeks before results can be reported to the owner. Because of these practical limitations, molecular diagnostic tools have been investigated to provide more immediate results.

Tapeworm diagnostics

Diagnosing anoplocephalid tapeworm infections remains a challenge despite the utility of several techniques. Fortunately, tapeworm diagnostic methods have been well-validated, so their weaknesses and strengths can be evaluated precisely.

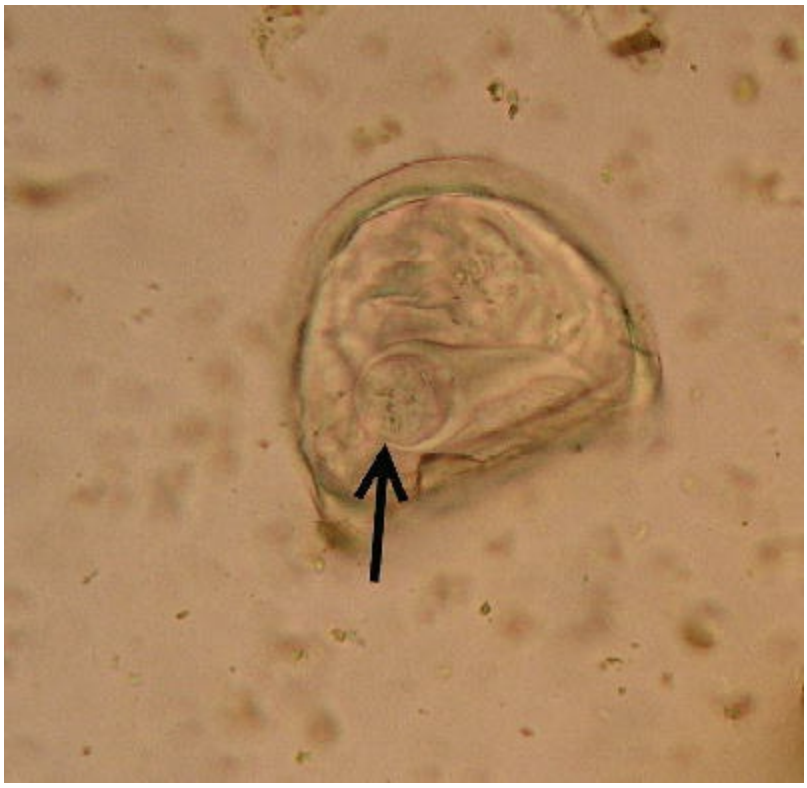
Fecal examination for tapeworm eggs

Any qualitative or quantitative fecal examination technique is capable of detecting tapeworm eggs (Figure 8.12). But, because cestode eggs are distributed unevenly in feces as proglottids disintegrate, the sensitivity of most techniques is too low to make a

reliable diagnosis. If eggs are found, they should be regarded as the tip of the iceberg, and many more could be recovered with a modified technique.

The basic modification employed for detecting tapeworms is to increase the amount of feces examined. Whereas a routine McMaster procedure typically uses 4 g of feces, 30–40 g of feces should be examined to increase the likelihood of finding tapeworm eggs (see Box 8.4 for description). Furthermore, the more effective tapeworm methods use enhanced flotation, which requires a centrifuge with a swing-bucket rotor to prevent cover slips from being dislodged. One study reported that using concentrated sugar (e.g., 1.26) as flotation medium yielded better results than saturated salt or zinc sulfate (Rehbein *et al*. 2011). A specific modification of the Stoll technique has been validated to achieve an overall diagnostic sensitivity of 61% (Proudman & Edwards 1992). Conversely, this method has a 39% chance of a false negative result, which is not overly impressive. However, these likelihoods were based on detection of cestode burdens comprising only a single worm. If the threshold were adjusted to detect 20 or more tapeworms, the sensitivity increases to 90% (Kjær *et al*. 2007; Proudman & Edwards 1992). In other words, only 10% of tapeworm burdens of 20 or more worms would be missed with this method. The choice of 20 tapeworms as a cutoff level is supported by the fact that no mucosal pathology is observed with tapeworm burdens of fewer than 20 worms (Bain & Kelly 1977; Pearson *et al*. 1993). Some studies have reported that examination of fecal samples 24 h after tapeworm treatment yielded higher *Anoplocephala* egg counts and a higher percentage of positive samples (Sanada *et al*. 2009; Elsener & Villeneuve 2011). Presumably, segments from dead tapeworms disintegrate within the host gut and release eggs into the feces.

Figure 8.12 Egg of *Anoplocephala perfoliata*. Eggs are often D-shaped and contain a distinct pyriform body (arrow). (*Source*: Photograph courtesy of Tina Roust and Maria Rhod)



In summary, the modified egg count method has proven useful for diagnosing moderate and large tapeworm burdens, while the smaller worm burdens may go undetected. The method is relatively easy to perform in most laboratories, but does require a centrifuge.

Box 8.4 Modified egg-counting method for detecting tapeworm eggs

This method is a modification of the Wisconsin technique, because it utilizes centrifugation-enhanced flotation. The key difference is the use of a larger fecal sample to increase the sensitivity of the method. Non-tapeworm parasite eggs will be encountered as well.

Materials

Disposable cups, tap water, wooden tongue depressor, cheesecloth (17 thread), disposable pipettes, 15 mL test tubes, flotation fluid with a specific gravity in the range of 1.018–1.025 (such as $ZnSO_4$, saturated salt, $MgSO_4$, saturated sugar-salt, or Sheather's sugar), laboratory balance with 0.1 g accuracy, microscope slides, cover slips (18 × 18 mm)

- Weigh 30 g of feces in a cup.
- Add 60 mL of tap water. Suspend the feces with the tongue depressor and rest for 30 min.
- Stir the suspension and pour through one layer of cheese cloth into another cup. Squeeze the cloth with the spatula to express all liquid into the cup.
- Pour the fluid into four 15 mL test tubes.
- Centrifuge for 10 min at 1000 g.
- Pour off the supernatant and preserve the pellet. Vortex the pellet (or stir with a wooden applicator stick) to resuspend it in the remaining fluid.
- Add flotation solution to about 3–5 mm below the rim of the tubes; stir well with wooden applicator stick.
- Centrifuge tubes for 5 min at 210 g.
- Gently add flotation solution to the tubes to create a slight meniscus.
- Place a cover slip on top of each tube and leave for 5 min.
- One can also fill the entire tube with flotation solution and centrifuge with the cover slips sitting on top of the tubes. But, this requires a centrifuge with a swing-bucket rotor to prevent loss of cover slips during centrifugation.
- Lift the cover slips off the tubes and place them on one or more microscope slides labeled with the respective horse's ID.
- Count all tapeworm eggs under the coverslip using the 4× or 10× objective.

Tapeworm serum ELISA

An ELISA method is currently available for detection of antibodies against 12/13 kDa excretory/secretory (ES) antigens of *A. perfoliata*. The ELISA has been thoroughly validated, with determination of diagnostic sensitivity and specificity as well as correlation with known worm burdens (Proudman & Trees 1996). However, interpretation of the results is not as straightforward as one might assume. A Danish abattoir study evaluating this assay found higher background levels of antibodies in *A. perfoliata* negative horses, which resulted in a high proportion of false-positive results (Kjær *et al.* 2007). Another study evaluated antibody levels following tapeworm treatment (Abbott *et al.* 2008) and found that horses remained antibody-positive for up to 5 months post treatment. In addition, recent data from horses co-infected with *A. perfoliata* and *A. magna* suggest a lack of specificity of the serum antibody ELISA (Meana *et al.* 2009).

These shortcomings indicate that antibody titers reflect exposure rather than contemporaneous infection. The serum ELISA remains a very useful test for investigating historical *Anoplocephala* exposure in a herd, but a positive result for an individual animal may not be a reliable indicator of current infection.

Diagnosis of Oxyuris equi

As mentioned previously, eggs of *O. equi* are usually not found in the feces due to the egg-laying behavior of the female worms. Irregular patches, ranging in color from light yellow to pale green and even orange, might be observed in the perianal area. A sample of this material usually contains numerous eggs, but pinworm eggs are often present on infected horses even if no visible deposit is evident.

Two methods are commonly employed to harvest eggs for microscopic examination. In the so-called "scotch tape technique," a

piece of cellophane tape is applied (sticky side down) to the perianal area and then transferred to a labeled microscope slide. Theoretically, perianal detritus, including *Oxyuris* eggs if present, will adhere to the tape and are captured for inspection. Alternatively, a wooden tongue depressor can be coated lightly with lubricant, and then used to firmly scrape the skin surrounding the anus. Material collected will adhere to the lubricant and can be transferred to a microscope slide and disseminated for visualization. *Oxyuris* eggs might be present in very low numbers, so detection with either technique may require careful microscopic examination.

Demonstration of microfilariae

Demonstration of the microfilarial stages of the genera *Onchocerca* and *Parafilaria* is not commonly attempted. However, antemortem diagnosis can be accomplished by biopsying a skin sample from an affected area, mincing it into very small pieces, and incubating the sections in saline. Microfilariae can be observed microscopically, swimming within the liquid medium (Klei *et al*. 2006).

Immunodiagnostics

Although many attempts have been made over the past 20–30 years to develop immunodiagnostic assays for the diagnosis of equine helminth infections, very few have been successfully validated. One example is the tapeworm ELISA mentioned previously, but assays targeting nematode parasites have been less successful.

Elevations of α- and β-globulin fractions and concurrent hypoalbuminemia were observed frequently in mixed strongyle infections (Schultze *et al*. 1983; Bailey *et al*. 1984). However, most protein measurements were found to be nonspecific and subject to high degrees of variation, with no pathognomonic pattern for strongyle infection (Bailey *et al*. 1984; Abbott *et al*. 2007). To date, no protein-based diagnostic assay has found application in veterinary practice.

A major component of the increased globulin fraction in equine strongyle infections is attributed to antibodies of the IgG(T) subgroup, which have been associated with *Strongylus vulgaris* infection (Patton *et al*. 1978; Kent 1987). This finding lead to the development of a commercial assay termed Aglutinade® Strongyle Test (Virbac), a latex agglutination kit measuring the level of IgG(T) in horse serum (Kent & Blackmore 1985). Although an association with strongyle infection was observed, other causes of IgG(T) elevation could not be ruled out because specificity of the antibodies was not evaluated. Thus, this assay was found to be of limited value (Klei 1986), and is no longer marketed. Subsequent attempts were made to develop specific serological assays for IgG(T) antibodies specific against *S. vulgaris*. Despite substantial efforts, cross-reactivity with other nematode species constituted a major obstacle to practical application (Klei *et al*. 1983; Weiland *et al*. 1991). To date, no satisfactory serological assay for the detection of *S. vulgaris* has been marketed.

Fasciola hepatica ELISA

A serum ELISA was developed recently for detecting antibodies to the liver fluke, *Fasciola hepatica*, in horses (Nelis *et al*. 2009). A positive correlation was shown between ELISA results and elevations of gamma glutamyl transferase (GGT), an enzyme that is regarded as a reliable indicator of bile duct pathology. Unpublished observations by one of the authors (MKN), however, did not find a similar association between GGT elevations and antibody titers using this assay. Further validation will be required before a useful test can be marketed to veterinary practitioners.

Molecular diagnosis

Molecular techniques have shown great potential for diagnostic purposes, but very few have progressed to routine usage. However, progress in the development of Polymerase Chain Reaction (PCR) instrumentation and other platforms for DNA amplification suggest that these methods will become cheaper and more user-friendly in the near future. Accordingly, some of these pending advances are discussed herein.

The diagnostic potential of gene sequences encoding ribosomes has been reported extensively (Campbell *et al*. 1995; Hung *et al*. 1999), and led to several rDNA-based PCR assays for detecting important equine parasites, for example, a PCR assay developed for the detection of *Anoplocephala perfoliata* DNA in fecal samples (Drögemüller *et al*. 2004). In a field study, however, it performed only slightly better than detection of eggs with the modified McMaster technique (Traversa *et al*. 2008).

A PCR-ELISA for identifying six species of cyathostomins has proven reliable and applicable for detecting the presence of these species in fecal samples (Hodgkinson *et al*. 2001, 2003, 2005). Similarly, a reverse line blot assay capable of detecting 13 species of cyathostomins and all three species of *Strongylus* has been developed and validated (Traversa *et al*. 2007). Recently, a real-time PCR assay has been applied for the detection and semiquantification of *Strongylus vulgaris* DNA in fecal samples (Nielsen *et al*. 2008).

Future diagnostics

Prepatent diagnosis of encysted cyathostomins

Several recent developments show distinct promise, and might become available in the relatively near future.

As mentioned in Chapter 2, encysted mucosal L_3 and L_4 are recognized as the main pathogenic stages of the cyathostomin life cycle, but coprologic methods cannot detect their presence. British scientists have isolated two antigens from encysted cyathostomins which offer potential for detecting prepatent worm burdens. Positive correlations have been reported between antibody titers and encysted worm burdens, which is also promising (Dowdall *et al*. 2004). Molecular investigations have further identified and characterized the protein component of one antigen which did not cross-react with luminal stages or with other helminth species (McWilliam *et al*. 2010). This body of work shows great promise, but additional studies are required before a diagnostic assay can be made available. Given the fact that all horses harbor encysted cyathostomins, it is important that such an assay be at least semiquantitative, differentiating between low, medium, and high numbers of encysted larvae.

Tapeworm coproantigen ELISA

Coproantigen ELISAs have shown some promise for detecting a number of helminth parasites. The basic premise is that antigens released by worms into the ingesta may be distributed more evenly than the eggs, which tend to occur in clusters. A coproantigen ELISA for diagnosing the equine tapeworm *Anoplocephala perfoliata* has been developed (Kania & Reinemeyer 2005) and validated, with 74% sensitivity and 92% specificity (Skotarek *et al*. 2010). This method therefore has potential to be applied in the field.

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9 Detection of Anthelmintic Resistance

Research with the parasites of other host species has resulted in the development and validation of molecular and *in vitro* assays for detection of anthelmintic resistance, but none of these has found application for horses. A major difference between the strongyles of horses and trichostrongylids of other livestock is the sheer number of cyathostomin species present in co-infections. It is typical to find 15–20 different species infecting one horse, which causes high variability in the test parameters evaluated.

The ability of benzimidazoles to kill developing larvae within a nematode egg has been exploited in an egg hatch inhibition test (EHT). Undeveloped eggs are isolated from fresh feces and incubated in varying concentrations of a soluble benzimidazole. The eggs of resistant worm strains are able to hatch at much higher concentrations than those from known susceptible isolates.

An EHT has recently been standardized for detection of benzimidazole resistance in ruminant nematodes (von Samson-Himmelstjerna *et al.* 2009), and could potentially be used for horses. However, the prevalence of BZD resistance in cyathostomin populations has reached a stage where testing for its presence is irrelevant. In addition, the EHT has not been validated for horses.

Larval development assays (LDA) have been validated for detection of anthelmintic resistance in ruminant trichostrongyles. The principle is to expose developing strongyle larvae to a range of drug concentrations, and evaluate the efficacy by assessment of dose–response curves. Attempts to validate LDA for diagnosis of cyathostomin resistance were confounded by excessive variability in the data, which caused too much overlap between susceptible and resistant strains of parasites, so the technique is not considered useful (Tandon & Kaplan 2004; Lind *et al.* 2005). Recent work with another *in vitro* method, the larval migration inhibition assay (LMIA), has shown promise for detection of ivermectin and moxidectin resistance in cyathostomins and may become available in the future (Matthews *et al.* 2011).

Molecular methods have been applied for detection of resistance, but these are hampered by the fact that relatively few genetic determinants have been identified. For benzimidazole resistance, single nucleotide polymorphisms (SNP) which confer resistance have been identified, and a real-time Polymerase Chain Reaction (PCR) assay has been developed for their detection (von Samson-Himmelstjerna *et al.* 2003). However, given the frequent occurrence of benzimidazole resistance, its mere identification has little value. It would be more important to have molecular tools available for early detection of emerging resistance, such as to ivermectin and moxidectin, but these have not yet been developed.

Despite all these elegant laboratory exercises, only one available technique constitutes a practical method for detecting anthelmintic resistance in horses: the fecal egg count reduction test (FECRT).

Fecal egg count reduction test

The underlying principle of the FECRT is extremely simple. The efficacy of an anthelmintic is evaluated by its ability to reduce fecal egg output after treatment. Fecal egg counts (FECs) are performed just before (or at the time of) treatment, and again 14 days after treatment, and the egg reduction is calculated for each individual horse according to the formula:

$$\%FECR = \left(\frac{FECpre - FECpost}{FECpre} \right) \times 100$$

Although this seems very straightforward, livestock parasitologists are presently involved in esoteric discussions to develop detailed guidelines to further validate the use of FECRT for detecting anthelmintic resistance in horses. Recently, a guideline committee has been formed under the World Association for the Advancement of Veterinary Parasitology (WAAVP). Horses comprise one of the focus areas of this effort, and more specific instructions can be expected in the future.

The following discussion presents recommendations that are based on the best information currently available.

Selection of egg-counting technique

Virtually any egg-counting technique can be employed for the FECRT, but a method with a detection limit of 25 or fewer eggs per gram is recommended. In cases of emerging resistance, post-treatment egg counts might be relatively low. If a technique with a higher detection limit were used, these few eggs may go undetected, and the FECR is calculated (inaccurately) as 100%. The egg-counting techniques with greater sensitivity tend to be more time-consuming, so the advantages and disadvantages of any technique must be considered, depending on the circumstances. Regardless of choice, it is important to use the same technique consistently pre- and post-treatment.

Table 9.1 Suitability of horses with different pre-treatment egg-count levels for inclusion in FECRT.

	Pre-treatment egg count			
Detection limit (EPG)	0–200	200–500	500–1000	>1000
1	++	+++	+++	+++
10	+	++	+++	+++
25	–	+(+)	+++	+++
50	–	+	++	+++

Depending on the detection limit of the egg-counting technique used, some egg-count levels may be too low for detecting reduced anthelmintic efficacy.
+++, very suitable; ++, suitable; +, avoid if possible; –, do not use.

The magnitude of the pre-treatment egg count is extremely important for the outcome of the FECRT. Let us consider an example in which a horse has a pre-treatment egg count of 100 EPG, determined by an egg-counting technique with a detection limit of 25 EPG. If the drug being tested is 85% efficacious, the "true" post-treatment egg count should be 15 EPG. However, because 15 EPG is below the detection threshold of the quantitative test, no eggs will be seen, and the post-treatment result will be reported (inaccurately) as 0 EPG, and efficacy will be calculated as 100%. If another horse with a pre-treatment egg count of 1000 EPG is treated with the same drug, it would be shedding around 150 EPG post-treatment. This number is well above the detection limit, so this horse's FECRT is calculated accurately as 85%. Therefore, FECRT efforts should preferentially use horses with moderate to high egg counts, especially when the egg-counting technique employed does not have a very low detection limit. Table 9.1 illustrates the relationship between egg-count ranges and FEC detection limits, and provides guidance for selecting horses and egg-count techniques for performing a FECRT.

Guidelines for diagnosing resistance

Anthelmintic resistance occurs at the parasite population level, and variability among horses may be extreme. Therefore, FECRT should be estimated at the farm level by determining FECR for a number of individual horses and then calculating the average FECR for the treated group. It is recommended that at least five to ten horses, each with FEC ≥ 200 EPG, be included from each farm, if possible. Test results from groups of fewer than five horses should be interpreted with great caution, unless very high efficacy (> 98%) or very low efficacy (< 80%) is seen consistently among all the horses tested.

In order to diagnose anthelmintic resistance, the expected efficacy of the drug being evaluated should be considered. Efficacies differ among various anthelmintics, and the reference standard can be found in the label claims for a product when it was first introduced to market. As an example, pyrantel formulations are typically 95%–100% effective, whereas ivermectin and moxidectin should have efficacies > 99%. Suggested efficacy values for indicating resistance depend on the drug class being evaluated, and the number of horses tested, but for the range of five to ten horses, the following guidelines should apply for equine strongyles:

Benzimidazoles	90%
Pyrantel	90%
Ivermectin	95%
Moxidectin	95%

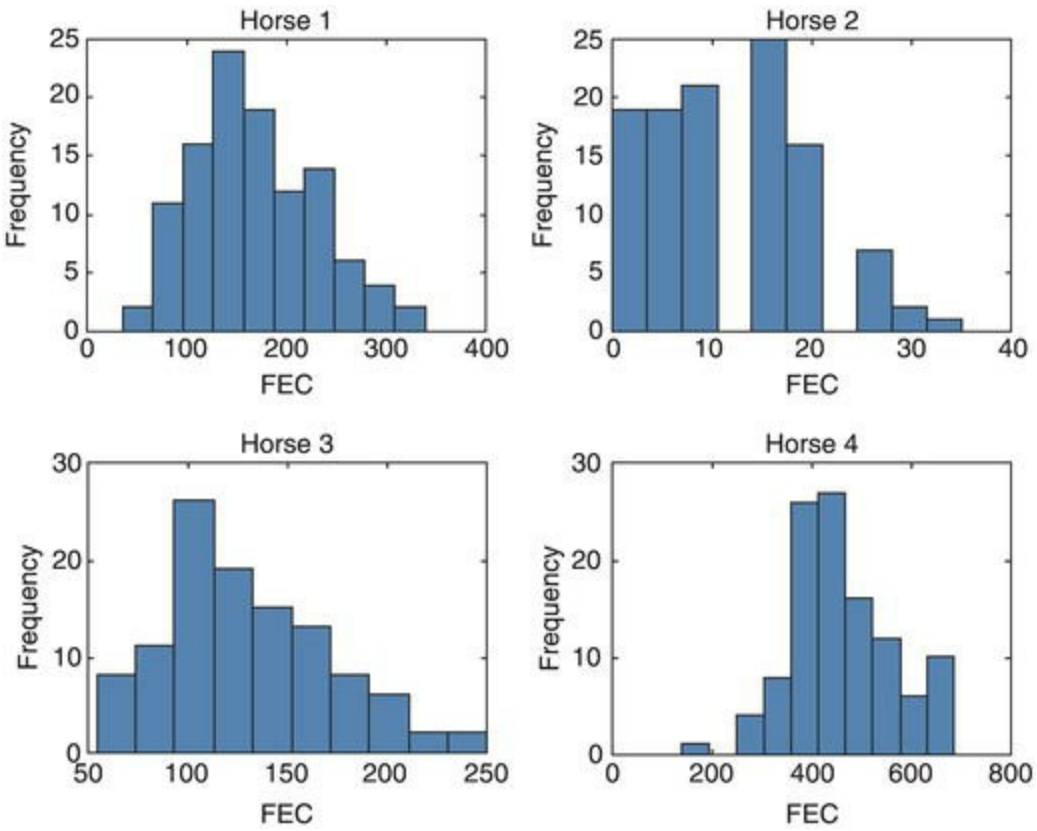
The same cutoff values can probably be applied for *Parascaris equorum*, although this assertion has not been confirmed by research. No available methods are capable of detecting anthelmintic resistance in equine tapeworms. The serum antibodies that are used for diagnosis remain elevated for months after treatment, and methods for counting tapeworm eggs are insufficiently sensitive for post-treatment follow-up (see Chapter 8).

Interpretation of FECRT

Although the mechanics of FECRT are relatively simple, interpretation of the results can be quite complicated. Egg-count data are notoriously variable, and it can be a challenge to take that variability into account when interpreting the results. As described in Chapter 8, individual egg counts vary in the range of ± 50%. In other words, each egg count should be viewed as one data point taken from a distribution with a certain mean and standard error. Unless we perform an infinite number of FECs from the same horse, we cannot know whether a certain egg count represents the true mean, or if it is closer to the upper or lower extremes of the distribution. In Figure 9.1, egg-count distributions from four different horses are presented.

One approach to establishing a more accurate estimate of the egg count for each horse is to perform two or three egg counts from the same fecal sample and calculate the mean value. Of course, this approach is laborious and therefore not likely to be performed in a busy veterinary practice. In addition to the variability among repeated egg counts for the same horse, an additional level of variability occurs between different horses on a farm. In the case of resistance, individual FECRT values will always fall within a wide range of values. Some horses may exhibit 100% efficacy, whereas others could show 70% or lower. This variability is reduced by always including several horses in FECRT and then calculating the mean value. In scientific studies, 95% confidence intervals of the mean are often calculated to establish a measure of the variability of the FECRT values (Coles *et al.* 2006). If the confidence intervals do not overlap with the predetermined cutoff values, this can be interpreted as a statistical indication of loss of efficacy (i.e., the variability exceeds that expected due to mere chance). It should be apparent that an estimate of anthelmintic efficacy will be more precise if more, rather than fewer, horses from a single farm are included in the FECRT.

Figure 9.1 Variability in the egg-count distributions of horses. The data in this figure represent 110 separate fecal egg counts (FECs) of the same horse, for four different horses. For each horse, five FECs were performed with each sample, and samples were collected approximately every 12 h over 11 days. Note the different magnitudes on the x-axis. (*Source*: Reprinted from *Veterinary Parasitology*, 185, Vidyashankar, A.N., Hanlon, B.M., Kaplan, R.M., Statistical and biological - considerations in evaluating drug efficacy in equine strongyle parasites using fecal egg-count data, 45–57, Copyright (2012), with permission from Elsevier)



If the farm average FECR falls below the cutoff values, anthelmintic resistance should be suspected. Given the variability described above, one should expect gray zones wherein the mean FECR is close to the cutoff values. In such cases, it is advisable to repeat the FECRT before resistance is concluded. Without calculating the confidence intervals, it is not possible to know when a certain result falls outside of the gray zone, but as a rule of thumb, results within 5% of the cutoff should always be interpreted with caution. If the sample is smaller than five horses per farm, the gray zone should be expanded to 10%. With pyrantel, for example, the gray zone would be 85–90%, and 80–90% if less than five horses are sampled. In general, FECRTs falling below the cutoff value should be reproducible. If a FECRT result cannot be reproduced, it was not resistance.

It is important to rule out other causes of decreased efficacy, such as intentional underdosing, partial loss of doses during administration, and reduced quality of drugs due to inappropriate storage. Establishment of accurate body weights is of paramount importance. If livestock scales are not available, the use of girth tapes is recommended. Because all current dewormers are administered orally, it is important to ensure that the entire dose is delivered and swallowed. Even though deworming is performed routinely and even rotely on many farms, it should be regarded as a significant therapeutic intervention. The consequences matter whenever 50% of the intended dose ends up on the stall floor.

Egg reappearance periods

A working definition for egg reappearance period (ERP) is "… the interval between an anthelmintic treatment and the time when parasite eggs can again be detected in the feces…." The ERP was originally introduced as a tool for designing suppressive treatment regimens, but it now also has application as a surveillance tool for detecting genetic changes in target populations. On one hand, a shortened ERP is evidence that treatment programs have selected for populations of worms which have shorter prepatent periods and reproduce more quickly than average. But in addition, it may also indicate developing levels of resistance.

The range of ERPs for a particular anthelmintic may extend from 2 weeks or less in a resistant isolate to the maximum interval identified when the drug was first approved for commercial distribution. ERPs that are intermediate within this range may

represent lower levels of resistance. Contemporary examples are the shortened ERPs of cyathostomins after ivermectin and moxidectin treatment, as described in Chapter 7. Table 9.2 presents reports of cyathostomin ERPs for different anthelmintic drug classes in populations with no signs of anthelmintic resistance. As a general rule, ERPs in susceptible populations should be more than 4 weeks for fenbendazole and pyrantel, more than 6–8 weeks for ivermectin, and more than 12–16 weeks for moxidectin.

Table 9.2 Cyathostomin egg reappearance periods (ERPs) given in weeks post treatment for different anthelmintic drugs in the absence of anthelmintic resistance.

Anthelmintic	ERP
Fenbendazole	6[a]
Pyrantel salts	5–6[b]
Ivermectin	9–13[c]
Moxidectin	16–22[7]

When anthelmintic resistance begins to develop, ERPs are considerably shortened.
[a]McBeath *et al*. (1978).
[b]Boersema *et al*. (1995; 1996).
[c]Borgsteede *et al*. (1993), Boersema *et al*. (1996), Demeulenaere *et al*. (1997).
[d]Jacobs *et al*. (1995), DiPietro *et al*. (1997), Demeulenaere *et al*. (1997)

Definitions

Although the general concept of ERP is relatively straightforward, multiple definitions or criteria for measuring it have been proposed. Some authors have defined it as the week of the first positive egg count post-treatment (Dudeney *et al*. 2008; Lyons *et al*. 2008). Others have used a fixed threshold for a mean egg count, such as 100 or 200 EPG (Boersema *et al*. 1996; Mercier *et al*. 2001). A third definition of ERP can be derived by performing FECRTs at weekly intervals post-treatment. The ERP is thereby defined as the interval when a calculated FECR falls below a predetermined cutoff value (Boersema *et al*. 1995; Tarigo-Martinie *et al*. 2001; von Samson-Himmelstjerna *et al*. 2007).

The definition that relies on the first post-treatment positive count unrealistically expects an initial FECR of 100%. An anthelmintic need not reduce egg counts by 100% to be considered fully efficacious. And, using a fixed threshold, such as 100 or 200 EPG, can be biased by the herd structure in terms of low-contaminator and high-contaminator horses. The low-contaminator horses will inadvertently take longer to reach a fixed threshold post treatment than horses with higher pre-treatment egg counts. An ERP definition based on FECRT calculation therefore represents a reasonable approach. The FECRT method considers the magnitude of pre-treatment egg counts, and the designated cutoff values can be tailored to the expected efficacy of the drug class being evaluated. A conservative approach is to set ERP cutoffs approximately 10% lower than the resistance cutoffs presented previously in this chapter. Thus, the corresponding values are as follows:

Benzimidazoles 80%

Pyrantel 80%

Ivermectin 85%

Moxidectin 85%

How to generate ERP information

When estimating ERP by the FECRT approach, standard considerations for the latter also apply (e.g., number of horses sampled, pre-treatment FEC levels, sensitivity of the egg-counting technique, etc.). ERPs are expressed, by convention, in units of "weeks post-treatment." Ideally, FECRTs should be performed weekly (after 2 weeks) until the cutoff value is reached, but that process is too laborious in a practice setting. A more pragmatic approach would be to target relevant time periods for the drug in question. For instance, the ERPs for pyrantel and fenbendazole are not expected to exceed 4 weeks, so ERP measurement would only be relevant at 3 weeks. Ivermectin and moxidectin have much longer ERPs, and it would be reasonable to begin screening at ~50% of the expected ERP. Thus, begin checking ivermectin at 3–4 weeks post post-treatment (expected ERP of 6–8 weeks), and moxidectin at 4–6 weeks (expected ERP 8–12 weeks).

It is important to emphasize that measuring ERP has no relevance once resistance has been detected. ERP surveillance, therefore, is currently more appropriate for tracking the efficacy of moxidectin and ivermectin against cyathostomins than for the other drug classes, in which resistance is more prevalent and operating at greater intensity.

Anthelmintic resistance in other parasites?

Although anthelmintic resistance has only been documented in cyathostomins and *Parascaris equorum*, the biological potential for resistance to appear in other equine parasites certainly exists (Reinemeyer 2012). Suspicions have been raised that some populations of *Oxyuris equi* were resistant to ivermectin, but a recent study demonstrated full efficacy of both ivermectin and pyrantel pamoate against adult and larval pinworms (Reinemeyer *et al*. 2010).

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10 Evaluating Historical Information

Parasitism is a common differential diagnosis for various clinical conditions of horses. However, the usual collection of history-generating questions is often inadequate for including or excluding parasitism as a definitive diagnosis. Clinicians are reminded that parasitism embodies the classic epidemiologic triangle of host, organism, and environment. Of these key factors, environment is the most complicated because it incorporates climate, habitat (facilities), and management interventions. Assessment of the likelihood of parasitic disease could be improved greatly if the anamnestic process were expanded to include environmental factors which influence the transmission of parasites.

Any number of approaches may be implemented for collecting an adequate parasitologic history for horses, and that process is best left to the experience of the practitioner. Regardless of personal preferences, the process should include information generated by asking the six classic questions learned by every journalism student: Who? What? When? Where? Why? How?

Who?

The "who" of our interrogatory process is the equine host. The specific subject of any history could be an individual horse, or a discrete herd under specific management circumstances, or even an entire farm comprised of multiple bands with varying management systems.

The host characteristic that has the single, greatest impact on the distribution of equine parasitisms is age. But, host age *per se* is usually just a surrogate parameter for other phenomena involved in a host/parasite interaction. Thus, *Strongyloides westeri* infections are seen only in suckling foals and recent weanlings, not because these animals are young, but because this organism is transmitted vertically from the dam to her offspring by the transmammary route (see Chapter 1). The unique age distributions of other parasitisms within a population actually reflect the development of acquired immunity. The prime example is *Parascaris equorum*, which is more prevalent in horses younger than 18 months of age than in older groups. Doubtless, older horses also ingest infective ascarid eggs on a continuous basis, but various immune processes prevent those exposures from culminating in mature parasites. Historically, pinworms have also been uncommon in mature horses, but this pattern may be changing (Reinemeyer 2012).

Host age can also help to prioritize differential diagnoses when it is compared to the known prepatent periods (PPPs) of potential parasitic pathogens. Thus, strongylid eggs observed in the feces of a 2-week-old foal cannot possibly indicate the presence of adult worms in the foal's gut because the minimum PPP for small strongyles is around 5.5 weeks (see Chapter 1). Similarly, strongyle eggs in the feces of a 4-month-old foal denote a reproducing cyathostomin population, but the eggs could not have been produced by large strongyles because the large strongyle PPP is greater than the host age.

Age also plays a significant role in the severity of damage inflicted by a parasitic infection. Adult horses are more resilient to parasitism than younger animals with less immunity, so the clinical effects tend to be much less severe in mature horses. Of course, this pattern can be modified by individual predispositions due to stress, immunosuppression, or concurrent disease.

At the extreme end of the age scale, no parasitisms are more prevalent in geriatric compared to mature horses. A healthy, geriatric horse in good flesh probably does not require more intensive parasite control than any normal, mature animal.

Sex of the animal is another host factor which may be an important variable for parasite susceptibility. Although little work has been done with horses, intact male sheep, goats, and cattle generally harbor significantly greater numbers of adult worms, have higher egg counts, and are more likely to suffer negative health consequences from a given type of parasite than intact females (Herd *et al.* 1992). Consistent with the pattern, neutered males are intermediate between the two sexes in their susceptibility to parasitism. This topic has great implications for stud farms, and deserves further practical research.

Although no breed predilections have been described for parasitisms of horses, minor differences exist between host species

within the family Equidae. For example, donkeys and burros are the usual definitive hosts of the equine lungworm, *Dictyocaulus arnfieldi*, but this nematode does not mature or reproduce well in horses (*Equus caballus*). Similarly, but of no clinical significance, a few cyathostomin species are recovered exclusively from donkeys, but apparently cannot infect horses grazing the same pastures (Tolliver 2000).

What?

For purposes of this discussion, we designate the historical "what" of parasitism as the various helminths which can infect equids. The likelihood of a specific parasite being the cause of a clinical problem varies with its prevalence, pathogenicity, host interactions, and details of its life cycle. Those factors were presented in Chapters 1 and 2, but are summarized in Table 10.1 for the major internal parasites of horses.

When?

"When" may be the most important question in the collection, and answering it requires detailed knowledge of parasite life cycles and epidemiology. The life cycles of most equine parasites are fairly protracted, and this is an important point to impress upon horse clients. The bots they see passing in the feces during spring actually began as eggs on the haircoat many months previously. And, even the core, scripted features of a life cycle can be prolonged beyond recognition by the vagaries of environmental persistence and arrested development. Thus, a new foal may experience a *Parascaris* infection even though the farm has not sheltered other juveniles for years, and individual horses can develop larval cyathostominosis despite several months of confinement and superior sanitation.

Strongyles and many other equine parasites are transmitted according to predictable, seasonal patterns. Knowledge of those patterns not only aids in predicting the times and circumstances of peak risk, but also helps to identify intervals when chemical control measures are unnecessary. Nearly every climate features long intervals when little parasite transmission occurs, and there is no need for treatment.

Time is an extremely important variable to consider when assessing the efficacy of control measures, and rigorous temporal guidelines for assessing fecal egg count reduction (FECR) have been presented in this book. As an example, deeming a dewormer ineffective because egg counts were moderately high at 6 weeks post-treatment is a false indictment in many cases (see Chapter 9). Temporal reconciliation of diagnostic results and historical information requires knowledge of the predictable declines in egg counts post-treatment, the expected duration of egg reappearance periods, and the quantitative variations that would constitute evidence of anthelmintic resistance.

Where?

Nearly all parasitisms can be transmitted on pasture, but only some can cycle in confinement. The hardy eggs of ascarids and pinworms are very self-sufficient and do not need to have a preference. Strongyles prefer pasture habitats, but third-stage larvae can be recovered from stall bedding, and doubtless some strongylid transmission occurs in confined horses. Tapeworms are transmitted exclusively on pasture, due to the essential role of free-living oribatid mites. However, one of the authors has observed cestode transmission in confined ruminants that were fed large bales of hay that had been stored outside. Soil mites apparently can invade hay bales stored in contact with the ground, and thus may be carried indoors to confined animals. Management sometimes confounds biology, and clinicians should always be on the lookout for exceptions to the rule.

Table 10.1 Prevalence, pathogenicity, host interactions, and life-cycle details influencing

clinical parasitism in horses.

Parasite	Prevalence	Pathogenicity	Host interactions	Life-cycle detail
Cyathostomins	Ubiquitous	Typically not very pathogenic	Disease is rare except when mass larval emergence occurs	Acquired immunity may modulate disease and egg shedding, but not infection
Large strongyles	Currently absent or inconsequential in many herds	Can be severely pathogenic	Various clinical signs from weight loss to fatal colic	Immunity may modulate disease, but does not preclude infection
Parascaris equorum	Ubiquitous; occurs almost exclusively in juveniles	Varies from no effect to fatal consequences	Weight loss, diarrhea, poor growth, intestinal obstruction	Acquired immunity ultimately controls all ascarid infections
Oxyuris equi	Ubiquitous; more common in immature horses	Negligible pathogens	Anal pruritus	Adult horses presumably develop immunity
Strongyloides westeri	Low prevalence in many herds	Modest pathogen	Most infections are asymptomatic; can cause watery diarrhea	Ultimately controlled by acquired immunity
Gasterophilus spp.	Ubiquitous	Minimal	Egg laying by adults is bothersome	Infections recur annually
Anoplocephala perfoliata	Ubiquitous in pastured horses	Varies from no effect to fatal consequences	Routine effects are unknown; serious consequences require surgery	Some horses may develop immunity

"Where" is a very reasonable question whenever horses develop a parasitic issue they have not experienced in the past. The simplest explanation for the appearance of a strange bug is that the hosts must have acquired it from a different environment. For example, investigation of lungworms as a diagnostic differential for pulmonary disease should include questions about historical exposure to venues with resident donkeys.

Although the examples are few in number, specific microhabitats have been identified as significant risk factors for certain parasitisms. Thus, damp sawdust bedding has been incriminated in clinical dermatitis ("frenzy") associated with *Strongyloides westeri* infection. The udder of a foaling mare is another specific location from which a suckling foal could acquire infective *Parascaris* eggs.

Why and how?

The "why" and "how" of parasitism are similar questions which basically reiterate life-cycle details and the modes of pathogenicity. But, numerous host and environmental variables become critically relevant when trying to explain why simple parasitism sometimes morphs into clinical disease. Constituent host factors have been addressed already in this chapter and in Chapter 4.

Environmental factors that have not yet been addressed include unique management features. We know that parasitic disease is a quantitative phenomenon, so one must consider any and all factors that could have resulted in a highly infective environment. The usual culprits include a high stocking rate on permanent pastures, particularly if grazed by young horses which tend to have higher egg counts than mature animals. Spreading uncomposted manure could also result in unusually high levels of infectivity.

Another contributing factor could be the administration of an anthelmintic which failed to reduce contamination for some reason. Potential explanations for treatment failures include under-dosing and anthelmintic resistance. Other scenarios are more complicated, such as administering a nonlarvicidal dewormer during a season when the majority of the cyathostomin population is in arrested development.

Other considerations

Although the classic journalism questions help to define the science of parasite history-taking, the art includes other factors which

are more difficult to characterize.

The significance of absence

The following conversation takes place in Conan Doyle's famous mystery story about the disappearance of a champion race horse (Silver Blaze):

"Is there any point to which you would wish to draw my attention?"
"To the curious incident of the dog in the night-time."
"The dog did nothing in the night-time."
"That was the curious incident," remarked Sherlock Holmes.

This literary episode demonstrates that valuable information can be deduced when something that should be present is inexplicably absent. For example, cyathostomins are ubiquitous in all horses except neonates, and even low contaminator adults are likely to be passing some eggs. Thus, the total absence of strongyle eggs in fecal samples from an entire herd is an unnatural situation, and the most likely explanations are effective anthelmintic treatment or lab error. So when treatment history is unavailable, but diagnostic results include a predominance of "0" egg counts, deworming is a feasible explanation.

On a related matter, the absence of eggs does not rule out the presence of worms, or even of parasitic disease (see Chapter 8). Indeed, this circumstance is commonplace in tapeworm infections, for which the sensitivity of all fecal diagnostic tests is notoriously low. Similarly, foals can harbor occult ascarid infections, or any horse may experience verminous arteritis, because both circumstances involve larval nematodes that are migrating systemically. These simply are not mature enough yet to begin laying eggs. The absence of a patent infection today is no guarantee of freedom from serious parasitic problems in a month or so (e.g., *Parascaris*), or even concurrently (larval cyathostominosis).

Do not over-interpret

It is important to recognize that most of the equine parasites listed in Table 10.1 are highly prevalent, so finding diagnostic evidence of their presence in a horse has few clinical implications. For instance, the passage of strongyle eggs by horses is extremely common, so finding eggs in the feces of a horse with colic symptoms should not be interpreted as comprising a causal relationship. Similarly, vague or unexplained clinical signs should not be attributed to *Anoplocephala* infection just because a serum ELISA from that horse revealed tapeworm-specific antibodies. The mere presence of a parasite is insufficient evidence to confirm disease, and certainly does not justify treatment. However, we appreciate that empirical treatment of an individual horse is a reasonable approach when definitive diagnosis is either impractical or expensive. Empirical treatment of entire herds, however, is a very bad idea.

A clinical diagnosis of parasitic disease can only be reached by considering all the information gathered during the interview process described previously. Diagnoses are typically reached by pattern recognition and an inclusion/exclusion approach. In the example of the colic horse that is shedding strongyle eggs, it would be relevant to note concurrent evidence of disease (e.g., unthriftiness, reductions in plasma protein and albumin, diarrhea) and to learn the interval since the most recent deworming. Like all infectious agents, parasites can contribute to any disease complex without being the primary cause.

Although gastrointestinal helminth parasites of livestock exist as populations distributed within a herd of animals, parasitic diseases rarely occur in epidemic form. The parasitologic picture of a herd equals the sum of the situations in multiple, individual horses, so a single animal may not accurately represent the typical situation within a host population. A single case of clinical disease does not indicate that the remainder of the herd is destined to develop the same signs. However, clinical disease does indicate that something is obviously suboptimal, which is an excellent reason to review the parasite management practices of the farm.

Lastly, it should be emphasized yet again that anthelmintic efficacy can only be evaluated in a population, and that FECRT should not be attempted with a single horse (see Chapter 9).

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11 Synopsis of Evidence-Based Parasite Control

When veterinary practitioners and horse owners turn to parasitologists for advice and guidance, the experience commonly results in frustration, annoyance, or wholesale rejection of any "new-fangled" recommendations. Those involved with the practical aspects of equine management typically seek simple and straightforward advice, and parasite control in horses has long been just that—a one-size-fits-all, generic recipe that only considers a drug and the calendar. Unfortunately, effective, sustainable, evidence-based parasite control is more complicated than most pragmatists suspect, and this book has attempted to explain why.

It is a very interesting exercise to ask clients, "Why do you deworm your horses?" Typical responses will touch on health maintenance, prevention of morbidity and mortality, and optimizing performance. But in addition, other, baseless reasons such as "tradition" and "it is a mandatory management procedure" always emerge if one continues to press.

At the core of evidence-based parasite control is a reason that is supported by scientific facts. So, first and foremost, it is of paramount importance to define the reason for, or the goals of, a parasite control program. Whether or not clients can verbalize what they are striving for, veterinarians must translate their objectives into Evidence-Based Parasite Control (EBPC) terms. Currently, the goals for equine parasite control can be defined as follows:

- To minimize the risk of parasitic disease
- To reduce infection pressure
- To maintain and prolong the efficacy of existing anthelmintics

Goals for parasite control have been revised during past decades, and they are likely to change again in the future as new knowledge emerges and new tools are developed. Inherent to pursuing these goals is the recognition of certain basic facts: (1) parasitism is a natural state of livestock and cannot be eradicated; (2) parasitic disease, or at least the negative impacts on health, cannot be avoided entirely, regardless of the control regimen implemented; and (3) control measures must be tailored to the conditions on each farm.

Considering the evidence

It should be unnecessary to mention that ignoring the evidence is the most hazardous approach of all. Nonetheless, this happens routinely in the realm of equine parasite control. A calendar-based anthelmintic treatment regimen, performed without surveillance of efficacy or a consideration of which parasites are present, systematically ignores most of the scientific evidence presented in this book.

It is impossible to make useful recommendations without detailed information about each farm and its control history. However, it is possible to present the key elements of a parasite control strategy, and those will serve as the scaffold on which a customized program can be assembled. In the following, we present several key elements that should exist in any parasite control program.

Measuring drug efficacy

As discussed in Chapter 8, the single most important reason for performing egg counts is to measure treatment efficacy by means of the fecal egg count reduction test (FECRT). The starting point for every farm is to evaluate the efficacy of what they are currently doing. Treatment with no assurance of efficacy creates a false sense of security, and populations of resistant worms can accumulate within the horses despite the labor and expense of misguided control efforts. As a rule of thumb, FECRTs should be performed on a yearly basis with each class of anthelmintic that was still effective last year. Details are presented in Chapter 9.

Basic treatment foundation

Parasite control programs are not unlike tract houses in any suburb. They differ in their details, but they all rest on a fairly similar foundation. In regard to horses, the foundation would be some control measure that is applied equally to all horses on a farm. Such measures are intended to minimize the prevalence of pathogenic parasites, and to limit the numbers acquired by susceptible foals and juvenile horses.

Data from Denmark, where anthelmintic treatments are limited to prescription-only use, suggest that large strongyle infections recrudesce if some horses on a farm are left completely untreated (Chapter 8). However, it does not require many anthelmintic treatments to suppress large strongyles. Given the long prepatent periods of *Strongylus* spp., one or two larvicidal treatments within the annual cycle should be sufficient to prevent transmission. Neither pyrantel nor a single dose of a benzimidazole will kill migrating larvae, so these are inappropriate for this purpose. The best time to apply such a treatment is toward the end of the grazing season. This will usually coincide with autumn in temperate climates, or during spring in warmer climates. At a maximum, treatments could be administered twice annually, at regular, 6 month intervals. No additional benefits would result from extra treatments.

As outlined in Chapter 2, the risk of parasitic disease is low in well-managed horses, but it is clear from the literature that the risk of developing disease is greater in young horses. *Parascaris equorum* is primarily a threat to foals and yearlings, and horses below the age of four years are at greater risk of developing larval cyathostominosis. These facts suggest that young horses require more intensive treatment than adults. The treatment needs of this age group depend on numerous factors, and should be customized for the management of each farm. Regardless, a few general rules can be followed to design control strategies for breeding farms.

Foals

As a rough guideline, most foals should have the benefit of approximately four anthelmintic treatments during their first 15 months of life. A greater number would require firm justification based on high infection pressure or clinical problems in the herd. Fewer than four treatments would be considered inadequate in most cases.

The major helminth pathogen in foals younger than 6 months of age is *Parascaris equorum*. Ideally, ascaricidal treatments should be timed as soon as possible after achievement of patency, but without frequent monitoring, it is impossible to determine such optimal timing. Therefore, the first anthelmintic treatment of foals is recommended around the age of 2.5–3 months. Considering the increased risk of impactions from paralytic anthelmintics (see Chapter 7), benzimidazoles may be the best choice at this time. A second deworming treatment should be targeted around or just before the time of weaning. This is a stressful period for the foal, and large parasite burdens would just compound the situation. The main parasitic threat at this time is still likely to be *P. equorum*, but strongyle parasites may start to play a role as well. Therefore, preweaning fecal egg counts yield useful information about the presence of ascarids and strongyles, and help guide the veterinarian in anthelmintic selection. Benzimidazoles are unlikely to be effective against cyathostomins, so other drug classes need to be considered. A third treatment for yearlings should be considered during the subsequent spring, when treatments for adult horses might be implemented concurrently. Drug selection at this time will depend on the results of yearly FECRTs performed on the farm. In areas with defined grazing seasons, a fourth treatment should be considered about midway through the grazing season. Yearlings are considerably more susceptible to parasitic infection than older horses, and yearlings could easily acquire large parasite burdens if left untreated for an entire grazing season of 5–6 months' duration.

Young horses

Treatment regimens will vary among farms for horses between 1 and approximately 4 years of age. Most programs resemble a compromise between a program appropriate for yearlings and one recommended for adult horses. Many farms would be justified to administer three treatments to this age group within an annual cycle. The three treatments could be scheduled before, midway, and toward the end of the grazing season. Cyathostomin larvicidal treatments using moxidectin or fenbendazole (where

efficacious) might be considered for the final annual treatment in this age group.

Other considerations

Control efforts against the tapeworm *Anoplocephala perfoliata* should be considered in most regions. Current diagnostic tools for tapeworm detection have clear limitations (see Chapter 8), so routine screening for this parasite in entire herds is rarely feasible. However, testing a representative sample of the horses for tapeworm antibodies will at least detect the presence of infection within the herd, and may reflect the level of tapeworm exposure. A pragmatic approach could combine tapeworm treatment with the foundation measures. Combination anthelmintics (praziquantel plus ivermectin or moxidectin) are ideal for this use. The best timing for cestode treatment is near the end of the grazing season, which is identical to the recommended scheduling for large strongyle larvicidal measures. If serum antibodies indicate high levels of exposure, an additional treatment within the annual cycle could be considered.

Incidental diagnoses may indicate that some herds would benefit from additional treatments to control nonalimentary parasitisms, such as cutaneous habronemiasis or onchocerciasis.

Farm strategies, adult horses

As outlined in Chapter 7, fecal egg counts can be used systematically to identify adult horses with consistently low, moderate, or high levels of strongyle egg shedding. Currently, there are no incentives for treatment of low egg shedders beyond the foundation treatments suggested previously. High egg shedders require additional attention, and daily, in-feed pyrantel or moxidectin could be considered for suppressing egg shedding more efficiently in these horses (Chapter 7). However, applying such treatments routinely to all horses in the herd has no biological or medical justification. Treating adult horses during the off-season is similarly unjustified if parasites were managed adequately during the active transmission season.

What is expected in the future?

Detailed advice has a limited shelf life because new knowledge will always force us to modify our recommendations in the future. The only reasonable accommodation is to make the best use of the knowledge presently at hand, and to remain open to new information and be willing to change our parasite management accordingly. Experience has shown us how difficult this can be, and the present book is just the most recent installment in a series of efforts to equip veterinarians and their clients with the tools and knowledge to make such changes.

Although prediction is impossible, we can identify some potential research developments that would convince us to adjust our recommendations. A few examples are presented in the following.

Improved diagnostic tools?

More accurate diagnostic tools would enable us to refine our strategies better. For example, diagnostic tests capable of quantifying burdens of nonpatent infections would be a superior indicator of the risk of potential parasitic disease in comparison to our current egg counts and larval cultures. Such tests would be extremely valuable for encysted cyathostomin larvae, migrating stages of *Strongylus vulgaris*, or prepatent *Parascaris* larvae. Similarly, an accurate and quantitative method for estimating tapeworm burdens would be seminal for finally developing evidence-based control strategies for *Anoplocephala* control. As outlined in Chapter 8, some recent developments in this field have been promising.

Last but not least, less laborious tests for detection of anthelmintic resistance would greatly simplify the present dual-sample procedures, and could remove much of the uncertainty with FECR calculations that fall in the "gray area" (see Chapter 9). Similarly, we cannot detect anthelmintic resistance in tapeworms at this time.

Risk of disease?

Very few epidemiologic studies have evaluated the risk of disease associated with various parasite burdens or different anthelmintic treatment regimens. Such studies are essential, and would likely lead to adjustments in our recommendations. The potential diagnostic tools for detecting migrating or encysted parasite larvae, as mentioned previously, would be extremely useful for conducting such studies in the future.

Changes of biology?

In addition to selecting for anthelmintic resistance, chemically based management programs likely have also selected for changes in other biological traits in the target populations. Such changes may include shorter prepatent periods or entire life cycles, adaptation to a different host-age spectrum, and changes in pathogenic potential. The history of parasitology abounds with examples of parasites that were initially considered harmless to their hosts, but subsequently found to be of potential clinical importance. For horses, cyathostomins and tapeworms comprise such examples. And we must remember that the historical standards of "harm" were tantamount to clinical disease. The tenets of modern production agriculture have shown repeatedly that pathogenic organisms need not cause obvious harm to result in economic loss, or to compromise host health through immunosuppression and other nonspecific mechanisms.

Further development of anthelmintic resistance?

Anthelmintic resistance is a natural and inevitable consequence of drug treatment. As long as chemical control remains a feature of parasite management, we will be selecting for resistance in target parasite populations. Increasing levels of anthelmintic resistance in the coming years are virtually assured. The first reports of total treatment failure against cyathostomins or *Parascaris equorum* may be just a few years away. Once this knowledge is publicized, the horse-owning public will demand radical changes, and the veterinary profession must be equipped to respond.

No biological rationale can assume that large strongyles are incapable of developing anthelmintic resistance too. The most likely explanations for its delayed appearance include longer life cycles, which protract the generation interval, and the high efficacy of larvicidal treatments. Anthelmintic resistance in a highly pathogenic parasite like *Strongylus vulgaris* would constitute a very serious threat to equine health.

New anthelmintics?

At some point in the future, the pharmaceutical industry will introduce new anthelmintic drugs to the equine market. However, these pending products will differ from their predecessors in many important ways. First, they are not likely to be as efficacious as the macrocyclic lactones. Drugs with such exceptional efficacy, safety, and breadth of spectrum only come along once in a generation. Second, new anthelmintics will be far more expensive than currently available dewormers, and manufacturers should give serious consideration to marketing these products on a strictly ethical basis, and forgoing over-the-counter sales. These measures should be implemented to get veterinarians more involved in parasite control programs, and if for no other reason, to dissuade casual abuse by horse owners (As of 2012, it is possible to purchase a dose of generic ivermectin paste to deworm a 500 kg adult horse for a retail cost of less than U.S.$3). Third, these products must be used more wisely and in a sustainable fashion. If new compounds are abused as blatantly as their ancestors, eventual resistance is assured. With this in mind, we can only hope that commercial manufacturers will change their advertising strategies to promote sustainable use and thereby support the longevity of their product in the market place.

Of course, incorporating new anthelmintics into customized control strategies will depend on a number of factors such as parasite spectrum (both species and stage of development), egg reappearance period, risk of adverse reactions, and cost of treatment. Regardless, the basic recommendations presented in this book are not likely to change just because a new drug has been introduced. Responsible use to maintain high efficacy will be paramount with any new or existing products.

Clinical cases for self-assessment

The 20 cases included in this book were carefully chosen to assess the reader's knowledge about equine parasites, and to illustrate and demonstrate the concepts presented in the previous chapters. Each case history is accompanied by three or more questions, and the suggested answers are provided. We recognize that a typical reader may not read this book from beginning to end, and may just select chapters or sections that are relevant to a current clinical challenge. A busy clinician might even prefer to start out with the cases, and we certainly welcome this approach. Regardless, should you find yourself struggling, each case will guide you to the relevant chapter for review. Enjoy.

Section IV

Case Histories

Case 1

Mystery Drug

History

A new client has a group of five Thoroughbred yearling colts that are being prepared for sale. Your partner dewormed this group about 3 weeks ago (~March 1), and she based her anthelmintic selection on the results of pre-treatment fecal exams. You recall that she had to go back the day after deworming to treat one of the colts for a mild colic.

The post-treatment FECR results have landed in your mail box by mistake, so you accept the intellectual challenge of figuring out which dewormer she used (Table C1.1) You might also try to deduce a thing or two about the resistance status of the herd.

Table C1.1 Pre- and post-treatment fecal egg count results following treatment with an unknown equine anthelmintic.

	Strongyles*		*Parascaris**		*Anoplocephala*†	
Horse ID	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
A	825	250	175	25	63	0
B	900	350	125	0	47	0
C‡	500	250	325	25	22	0
D	1275	375	250	0	103	0
E	450	100	250	0	7	0

* McMaster; sensitivity 25 EPG.
† Modified Wisconsin technique, 1 EPG.
‡ The lab record noted that two bots were found in the feces when submitted.

Questions

1. Which anthelmintic did she use? Can you deduce anything else about the anthelmintic susceptibility status of this herd?

2. What do you make of the bot stragglers?

Answer

1. Let us go through the options in an organized fashion, and rule in or rule out the various classes.

Benzimidazoles: The treatment failed to achieve ≥90% efficacy against strongyles, so cyathostomin resistance must be a feasible characteristic of the mystery drug. Small strongyle resistance to BZDs is extremely common, plus the treatment was effective against adult ascarids. These features both support the historical use of a BZD such as fenbendazole or oxibendazole. However, the BZDs have no efficacy against tapeworms, so we can assume that this drug class was not used.

Macrocyclic Lactones: As long as the target population is not ML-resistant, this class generally exhibits good efficacy against ascarids. However, ivermectin or moxidectin have no cestocidal properties, so this class cannot explain the removal of tapeworms. But wait! There are….

Macrocyclic Lactones Combined with Praziquantel: Praziquantel (PRZ) is an extremely effective cestocide, and several combinations of PRZ plus ivermectin or moxidectin are commercially available. The use of one of these products would explain the observed efficacy against ascarids and tapeworms, but ML resistance to the extent exhibited in these strongyle egg counts has not been reported, and hopefully is many years away.

Pyrimidines: The pyrantel salts are broad-spectrum dewormers, so they exhibit good efficacy against strongyles, ascarids, and pinworms. The mystery treatment did a good job against *Parascaris*, but the removal of strongyles was inadequate, and likely indicates resistance. This is quite feasible because pyrantel-resistant cyathostomins were detected in nearly half of several herds surveyed in the U.S. within the past decade. But, does pyrantel have any efficacy against tapeworms? Indeed, one pyrantel pamoate paste formulation is labeled for efficacy against *Anoplocephala perfoliata* when administered at 13.2 mg/kg. At the standard nematocidal dosage (6.6 mg/kg), pyrantel pamoate removed 80% or more of adult cestodes in controlled efficacy trials (see Chapter 9).

So, you have not only identified the mystery drug, but you also recognize that the herd in question probably harbors pyrantel-resistant cyathostomins. Also, ascarid impactions or obstructive colics are more common after treatment with anthelmintics that have a neuromuscular mode of action (see Chapter 7), so this item of historical trivia is also consistent with your product identification.

2. The bots were probably passing out of the host as a normal, seasonal phenomenon. Three weeks post-dosing is far too late to be a consequence of treatment. And the fact that bots are still present 3 weeks after treatment is further evidence that macrocyclic lactones were not used.

Case 2

Pyrantel Efficacy Evaluation

History

A horse owner has sought your assistance to test for anthelmintic resistance against a pyrantel paste formulation on his Standardbred farm. He has used this drug regularly over the past 10 years, and now wants to determine whether resistance has developed. You perform a fecal egg count reduction test (FECRT) and generate the data presented in Table C2.1.

Table C2.1 Strongylid fecal egg counts from a Standardbred herd, measured before and 14 days after treatment with pyrantel pamoate paste.

Pre-treatment	Post-treatment
300	0
300	20
780	0
520	0
340	0
400	60
260	0
280	40
860	140
400	80
300	0
200	120
300	20
640	40

Questions

1. What is the calculated FECRT for this farm?

  a. What is your interpretation of the result?

2. What is your recommendation for this farm regarding the future use of pyrantel products?

Answers

1. The FECRT is calculated in Table C2.2, using guidelines presented in Chapter 9. Fecal egg count reductions are calculated individually for each horse, and the mean value is obtained for the farm. In this case the FECRT is 89.63.

a. This result is inconclusive. Although the FECR is numerically below 90%, these results fall in the gray zone, wherein the influence of chance variability cannot be excluded. Therefore, it is recommended that the FECRT be repeated for this farm.

2. Horses would benefit from a 90% reduction of egg shedding, so the drug can be used on the farm. However, if the true efficacy is only 90%, resistance may be accelerated if this drug is used frequently or exclusively. Recommendations would be to retest pyrantel's efficacy, as mentioned above, and possibly use a drug with a higher efficacy.

Table C2.2 Calculation of FECRT results for the farm.

Pre-treatment	Post-treatment	FECR (%)
300	0	100
300	20	93.33
780	0	100
520	0	100
340	0	100
400	60	85.00
260	0	100
280	40	85.71
860	140	83.72
400	80	80.00
300	0	100
200	120	40.00
300	20	93.33
640	40	93.75
Total FECR		**89.63**

Case 3

Egg Count Results From Illinois Yearlings

History

A herd of pastured yearlings from northern Illinois was screened for possible enrollment in an anthelmintic field trial. Farm employees were not familiar with any details regarding recent anthelmintic treatment, but those records could be supplied by the stable manager when he returned from vacation. The following fecal diagnostic results were reported (Table C3.1).

Table C3.1 Quantitative fecal egg count results, Big Ridge Stables.

Horse ID	*Parascaris**	Strongyles*	*Anoplocephala**
A	456	0	17
B	1019	0	3
C	0	0	0
D	177	0	25
E	342	0	137
F	556	0	88
G	412	0	76
H	30	0	0
I	601	0	33
J	18	0	60

*All results reported as eggs per gram.

Questions

1. How can one determine that the quantitative fecal examination procedure used was NOT the McMaster's technique?

2. What are the two most unusual features of these fecal results?

3. Of these two unusual features, which is clearly a consequence of pasture-based management?

4. What is the only explanation for the other anomaly?

5. The prevalence and magnitude of ascarid egg counts allow one to define the *specific* management intervention that was identified by question #4. What was it?

6. What future recommendations might you provide for helminth management in this herd?

Answers

1. The McMaster's is a dilution technique in which the raw egg counts (*numbers of eggs actually observed microscopically and counted*) are multiplied by a standard conversion factor to generate final egg counts (*expressed as eggs per gram*). For example, a McMaster technique using 4 g of feces and 26 mL of flotation solution has a standard conversion factor of 25. Thus, all of the positive egg counts from a similar McMaster technique would be divisible by 25. The egg counts in this series have no common denominator >1.

2. (a) Unusually high prevalence of positive results for tapeworms (*Anoplocephala*).

(b) Total absence of positive strongyle egg counts.

3. *Anoplocephala* infections are transmitted by ingestion of free-living oribatid mites, which occur almost exclusively in forage-based management systems.

4. It is virtually unheard of for an entire pastured herd of young horses to have strongyle egg counts of "0." The only logical explanation is recent treatment with an anthelmintic that exhibits high efficacy against strongyles.

5. If anthelmintic therapy is the only explanation for consistent "0" strongyle egg counts, then treatment must have involved a drug that concurrently spares tapeworms and ascarids.

One can rule out any anthelmintic combination containing praziquantel, which has virtually 100% efficacy against cestodes (plus, the prepatent period for *Anoplocephala* is much longer than the reappearance period of strongyle eggs). One could probably also exclude any treatment involving pyrantel products because they also have some efficacy against cestodes. In addition, pyrantel would be unlikely to reduce strongylid egg counts to zero in all treated horses.

This leaves only benzimidazoles, piperazine, and macrocyclic lactones as alternative explanations. Piperazine exhibits good efficacy against ascarids, but only modest activity against strongyles. The benzimidazoles and macrocyclic lactones are both broad-spectrum drugs, with efficacy against ascarids and strongyles. In this case, the operative drug clearly worked against only a single target population—strongyles. How can one explain the lack of efficacy against *Parascaris*?

The farm manager was contacted relative to these results, and he was able to confirm that all yearlings had been dewormed 2 weeks previously with ivermectin paste. Clearly, the ascarid population on this farm was resistant to macrocyclic lactone anthelmintics, although the indigenous strongyles were obviously susceptible.

6. See Chapter 11, for a discussion of ascarid management in herds with known macrocyclic lactone resistance. The high prevalence of *Anoplocephala* eggs indicates that regular cestode control measures are warranted. Biannual treatments (spring and autumn) with praziquantel or pyrantel pamoate (13.2 mg/kg) should be sufficient.

(*Note*: In the authors' experience, a high prevalence of patent *Anoplocephala infections in a herd may indicate the presence of A. magna* or mixed infections, rather than *A. perfoliata* alone. Management recommendations would be identical.)

Case 4

Colic and Parasites

History

An 8-year-old Icelandic stallion used for breeding and competition riding collapsed at an event with signs of colic. A veterinarian present at the event examined the horse, and found it in lateral recumbency with cyanotic mucous membranes and a heart rate of 80 bpm (beats per minute). She referred the stallion to a university hospital for further management. Following admission, the signs of pain could not be controlled, and an exploratory laparotomy was performed. Surgeons found a stricture of the dorsal colon, with fibrous adhesions to the abdominal wall. The adhesions were broken down and the stricture was resected. Recovery was slow but uneventful.

The owner was informed that the lesion could have originated as parasitic damage. She admits that her horses had not been dewormed at all during the past year. She owns a small stud farm and is now worried that her horses might suffer from serious parasite infection. None are showing any signs of parasitism, but she asks her local veterinarian to collect fecal samples from all horses on the premises and perform fecal egg counts and larval cultures. The results are presented in Table C4.1. Figure C4.1 depicts some of the noncyathostomin larvae that were observed.

Table C4.1 Results of fecal egg counts and larval cultures for all horses on a farm.

Larval culture results are presented as the total number of noncyathostomin larvae encountered in the sample.

Age of horse	Fecal egg count	Noncyathostomin larvae
16	40	0
18	0	4
10	20	0
5	240	6
10	380	5
4	1000	26
21	120	0
1	160	0
1	2300	121
2	960	37
4	200	2
2	440	6
31	1340	3
14	640	54
8	180	0
1	1600	27
10	60	0
10	120	1
7	0	0

Figure C4.1 Non-cyathostomin larvae encountered in larval cultures (arrows)

Questions

1. Which strongyle species do you recognize in Figure C4.1?

2. Which anthelmintic drugs would you expect to be efficacious against these parasites?

3. What anthelmintic treatment strategy do you recommend on the farm?

Answers

1. The smaller larvae in the picture are cyathostomins, and are characterized by filamentous tails and eight intestinal cells. The larvae indicated by arrows are *Strongylus vulgaris*. They are larger than the cyathostomin larvae present, and definitive identification is provided by the presence of 28–32 intestinal cells.

2. All broad-spectrum equine anthelmintics have good efficacy against adult *S. vulgaris*. However, they are not all effective against migrating stages. The macrocyclic lactones are effective and work against all stages, and fenbendazole can kill migrating larvae, but only if administered at an elevated dosage for 5 consecutive days. Pyrantel only has activity against luminal stages.

3. Several options are available for this farm, depending on their goals. In this case, the most important goal is to reduce the presence of *Strongylus vulgaris* to a negligible level. The owner must recognize that eradication is not feasible, but that large strongyles can be reduced to a level where they are encountered in low numbers in just a few horses. Another relevant goal would be to reduce egg shedding for the entire farm.

It would be relevant to discuss management procedures with the owner, especially the quality and availability of pastures. If the owner is motivated, pasture hygiene can be recommended to reduce the infection pressure.

For anthelmintic treatments, the basic foundation should include larvicidal applications that are sufficiently frequent to disrupt the life cycle of *S. vulgaris*. As the prepatent period is about 6 months, two treatments, spaced at equal intervals during the annual cycle, should be sufficient. Macrocyclic lactones at 6 month intervals would be very efficient, and moxidectin could be used preferentially over ivermectin for horses shedding more than 1000 EPG (to provide a longer egg reappearance period). Egg counts should be evaluated at least once annually to identify the high,

moderate, and low egg shedders. Larval cultures can be performed in the spring at the onset of the grazing season to detect large strongyle infections.

A few additional treatments should be considered for foals that are at risk of acquiring *Parascaris equorum* burdens.

Case 5

Confinement after Deworming

History

You are contacted by the owner of a riding school with 80 adult horses and ponies. For many years, they have practiced meticulous, regular deworming of the entire herd. As a routine post-deworming procedure, all horses are held in their stalls for 5 days before being turned out to pastures. During this confinement period, personnel remove all manure and replace the bedding. This measure is labor-intensive and expensive, so the owner wants to know whether shortening the period to 3 days would present any risks.

Questions

1. Identify the risks of parasite transmission, if any, during the post-treatment period. Which parasites are in question, and why are they a risk during the post-treatment period?

2. Describe potential adverse reactions to anthelmintic treatment that any horse might experience. What is the likely post-treatment interval during which most of these adverse events would occur?

3. Considering your answers to questions 1 and 2, how would you respond to the owner's query about an abbreviated interval?

Answers

1. The rationale behind this confinement procedure is unclear, and is not really very logical. The primary parasites in adult horses are strongyles, and possibly tapeworms and pinworms. *Parascaris equorum* is unlikely to occur in mature horses. It is important to remind the client that infective stages develop from eggs that are passed in the feces. Horses with positive egg counts shed thousands of parasite eggs every day, and this contamination gradually declines to a very low level during the days following an efficacious treatment. Daily removal of feces would eliminate the source of eggs before they could develop, and the presence of anthelmintic metabolites (especially benzimidazoles and macrocyclic lactones) would likely interfere with successful translation. Dead worms are always expelled after deworming, but only the larger of these are visible to the naked eye.

Tapeworm eggs have been found to be more abundant in the feces following treatment with a tapeworm drug in horses (see Chapter 8). However, it is important to remember that the life cycle involves development within oribatid mites before horses can become infected. It is unknown whether these mites occur in stalls, but the risk of tapeworm infection in stables must be considered minor. The only other possible justification for this practice is the theoretical risk of eggs contained within the uteri of treated female worms being able to develop into the infective stage. For strongyle parasites, this must be considered unlikely because a majority of these eggs would be immature and not yet fully developed. In addition, the worm would have to disintegrate for the eggs to make it to the environment, and daily removal would obviate that risk as well.

The best theoretical justification for the confinement procedure would be for *Parascaris equorum* in foals. A female

ascarid contains hundreds of thousands of eggs. Given their ability to resist environment influences, a share of these eggs are likely to remain viable even after the death of the worm. However, the riding school in question only has adult horses.

2. Post-dosing reactions involve nonspecific colics, transient diarrheas, and larval cyathostominosis. Although these are more likely to occur in younger horses, chances are that some of the 80 horses at this riding school may encounter such reactions. It is therefore advisable to monitor horses for these reactions. However, this does not necessarily involve stall confinement. The choice of observation period is also unclear. Most of the milder reactions occur within the first few days, but one study showed that anthelmintic treatment constituted a risk factor for larval cyathostominosis during the following 2 weeks.

3. As outlined above, this confinement procedure makes very little sense, and we see no reason to continue it. Disinfection measures are not justified, and are generally ineffective anyhow. At most, the owner and her personnel should be instructed about the small risk of adverse reactions to anthelmintic treatment.

Case 6

Abdominal Distress in a Foal

History

A 5-month-old Trakehner filly was orphaned at birth and hand-raised in confinement. The foal had received tetanus antitoxin after birth, but no additional vaccines or anthelmintic treatments in the interim.

In late August, the foal developed colic of 5 h duration. The referring veterinarian classified the pain as severe, and treatment with opioid analgesics provided only transient relief. The foal was referred to a tertiary care center.

Clinical assessment

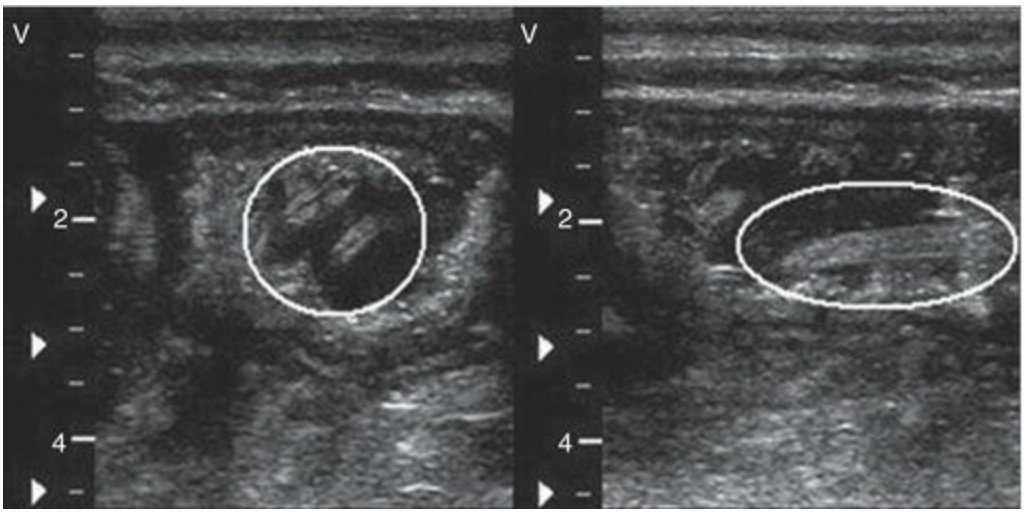
The foal presented with abdominal distention; mucous membranes were hyperemic with a CRT of 3 s, heart rate 40 bpm, and respiratory rate 20 bpm. Manifestations of colic were manageable with xylazine and metamizol, and gastric reflux was absent. Abdominal ultrasound revealed a distended small intestine with hyperechogenic, linear, motile objects within the lumen (Figure C6.1).

Laboratory findings

Hemogram	Normal
Serum chemistry	Unremarkable
Abdominocentesis	10 g protein/L and $<10^9$ leukocytes/L

McMaster quantitative egg count—480 strongyle EPG, 80 *Parascaris* EPG.

Figure C6.1 Transabdominal ultrasound of the small intestine of a foal with colic. Several hyperechogenic, linear objects were identified in the lumen (circled). The figure depicts transverse (left) and longitudinal (right) sections of the intraluminal objects



Questions

1. What is the most likely clinical diagnosis?

a. Other differential diagnoses?

2. What specific, antiparasitic treatment plan would you recommend for this foal?

3. The owners operate a small Trakehner breeding operation. What general recommendations would you make to prevent similar recurrences in future foals?

Answers

1. Small intestinal impaction with *Parascaris equorum*.

a. Other causes of small intestinal obstruction, such as volvulus or epiploic entrapment.

2. The foal was treated with one dose of fenbendazole paste (7.5 mg/kg) to minimize the risk of exacerbating the impaction (see Chapter 7 for a discussion of anthelmintics with a paralytic mode of action). The label dosage of fenbendazole (FBZ) for removal of ascarids is 10 mg/kg. The 7.5 mg/kg dosage should reduce ascarid numbers without creating a huge bolus of dead worms. Treatment with a full ascaricidal dosage of a benzimidazole should be repeated within a few days of the initial dose. Ivermectin and moxidectin may also be useful for this purpose, if these drugs have been proven efficacious on the farm.

Additional clinical information

In addition to fenbendazole, mineral oil was administered via nasogastric tube. The foal was monitored closely for 24 h after FBZ treatment, and evaluated for gastric reflux at 3 hour intervals. Approximately 40 dead ascarids were recovered via gastric intubation, and worms were observed passing in the feces from the second day following treatment. The foal was sent home 5 days after admission.

3. It is advisable to deworm foals at about 2–3 months of age to prevent accumulation of large burdens of *Parascaris equorum*. In consideration of the current levels of resistance to ivermectin/moxidectin and the risk of impaction associated with paralytic anthelmintics, benzimidazoles may be the best choice for this treatment. Fecal egg counts have little or no diagnostic value for foals that are younger than the prepatent period of *Parascaris*, but can be useful for evaluating treatment efficacy in older juveniles (see Chapter 8 for a discussion of diagnostic methods).

Case 7

Quarantining Advice

History

A client just bought a new, adult mare and asks for your advice. She owns a small, Arabian breeding operation and does not want to introduce "bad parasites" into her herd. She reports that clinical parasitism has never been a problem on her property, and she intends to keep it that way. Her initial plan is to quarantine the new horse on an isolated paddock before introducing it into the herd, but she has a couple of questions for you: (1) Which dewormer should she use for the new horse? (2) How long should she quarantine the horse?

Questions

1. Before attempting to answer her questions, she needs to define the potential goals of the intended quarantine. Which "bad" parasites should she be intent on avoiding?

2. For each of these, how would you prevent their introduction onto the farm?

3. Based on these requirements, do you concur with her desire to quarantine the horse?

Answers

1. The point of any quarantine measure is to prevent the introduction of only those parasites that are currently absent from the farm. Even without performing any fecal examinations, one can safely assume that her herd already harbors cyathostomins. *Parascaris equorum* is likely to be present in the foals and yearlings in residence, and *Anoplocephala perfoliata* would not be unexpected for a pasture-based management system. In general, two parasite categories are considered undesirable on any farm: (a) large strongyles, especially *Strongylus vulgaris*, because of its pathogenic potential, (b) drug-resistant populations of cyathostomins and/or *Parascaris*.

2. The large strongyles are relatively simple to manage, because anthelmintic resistance is not an issue with this group. For effective management, one should choose drugs with efficacy against both luminal and migrating stages, which rules out piperazine, pyrantel, and single-dose fenbendazole.

Avoiding anthelmintic resistance is much more difficult. Fecal egg count reduction testing (FECRT) permits evaluation of only one drug at a time, and is best performed when a horse is shedding moderate to high numbers of eggs. Especially relevant to the current case, FECRT is of limited value when performed on only one horse, and the results should be interpreted with great caution. The most pragmatic approach for quarantine is to test the efficacy of whichever drug is used most commonly on the farm. In most cases, this will likely be ivermectin.

3. Quarantining has the most value if one possesses solid information about the current parasite status of the farm. For instance, if large strongyles were already present, eliminating them from a new arrival is not a great concern. Similarly, knowing the efficacy status of various anthelmintic classes against indigenous parasites helps to determine the best diagnostic information that can be gleaned from the new horse.

Case 8

Diarrhea and Colic

History

A 7-month-old grade colt, 215 kg, was dewormed with an unknown product 2 months ago. Since that time, the foal has lost weight and developed a rough haircoat and "tucked up" appearance. Feces were soft to watery and preputial edema was observed. When admitted on January 1, the foal had experienced recurrent, mild colic for the past 4 weeks. The referring vet had been called to examine the colt on several occasions, and had treated it with opioids for pain, sulfa + trimethoprim antibiotics, and nonsteroidal anti-inflammatory drugs.

Clinical assessment

The foal had clear manifestations of pain, with constant rolling. The heart rate was within normal range, and mucous membranes were pink with a capillary refill time of 3 s. Transabdominal ultrasound revealed an abnormal appearance of the cecum with unusual, hyperechogenic areas. Feces ranged from loose to normal in consistency.

Laboratory findings

WBCC Neutrophils Total protein Albumin Abdominal tap	20.07×10^9 cells/L (leukocytosis) 17.32×10^9 cells/L (absolute neutrophilia) (86.3%) 38.98 g/L (hypoproteinemia) 22.38 g/L (hypoalbuminemia) 0.55×10^9 cells/L with protein 2 g/L (normal)	
Blood and feces for *Lawsonia intracellularis* Bacterial cultures for *Salmonella* and *Clostridium* spp. McMaster quantitative fecal egg count Direct Baermann for cyathostomin larvae		Negative Negative 40 strongyle EPG Negative

Treatment

The clinicians had no primary suspicion of a parasitic problem and were focused on managing the colic. Pain could not be controlled with the panel of analgesics available, so a laparotomy was performed on January 5. The cecum had an invagination involving ~50% of the organ wall. The affected portion of the cecum was resected, and the intestinal wall was found to be extremely edematous. Regional lymph nodes were markedly enlarged. After surgery, the colt was treated with intravenous fluids, lidocaine infusion, meloxicam, metronidazole, cefquinome, and omeprazole.

Questions

1. Which parasitic condition is the most likely cause of the case described herein? Which factors support this diagnosis?

a. What diagnostic measures can be applied to reach a definitive diagnosis?

2. Which anthelmintics should be considered for managing this condition?

3. What other equine parasite could cause the surgical condition described above?

Answers

1. Larval cyathostominosis should be suspected because of the following factors: the time of year, immature age of the host, historical loss of weight and body condition, loose feces, ventral edema, hypoproteinemia, leukocytosis, and a history of deworming prior to the onset of clinical signs. It is somewhat unusual to find this condition in foals, as they generally do not graze extensively during their first summer, and acquire relatively small burdens of encysted worms. However, larval cyathostominosis has been reported in weanlings. The bacterial infection *Lawsonia intracellularis* is an important differential diagnosis in this age group, and can cause similar signs. Some case studies have reported intussusceptions and cecal invaginations associated with larval cyathostominosis.

a. No reliable diagnostic tests are available for diagnosing larval cyathostominosis. The direct Baermann method employed here represents an attempt to identify recently emerged cyathostomin larvae in the feces, but this procedure probably has low diagnostic sensitivity. This is discussed further in Chapter 8.

2. Moxidectin should be considered for treating a clinical case of larval cyathostominosis (see Chapter 7). This drug appears to cause minimal inflammatory reaction and its efficacy against encysted cyathostomins is not compromised by potential resistance issues.

3. Tapeworms (*Anoplocephala perfoliata*) can cause spastic and mechanical colics. The latter can be attributed to ileal impactions, cecocolic intussusceptions, and, possibly, cecal invagination. Tapeworm infection causes inflammation and edema of the cecal wall, but lesions are generally restricted to the immediate vicinity of attachment sites. However, hypoproteinemia and hypoalbuminemia as observed in this case have not been associated with tapeworm infection.

a. A modified McMaster technique can be applied for *Anoplocephala* diagnosis. An ELISA technique for detecting antibodies is also an option, but a positive result may indicate historical exposure rather than contemporaneous infection (see Chapter 8).

Outcome

After initially improving for approximately 2 weeks post-surgery, colic signs resumed and the horse was euthanatized on February 1. At necropsy, regional parasitic typhlocolitis (i.e., larval cyathostominosis) was diagnosed, and myriad, vacated, parasitic cysts were visualized in the mucosal lining, along with marked edema of the large intestinal walls and enlarged regional lymph nodes.

Case 9

Foal Diarrhea

History

A Morgan horse breeding facility foaled approximately one dozen mares this spring. The mares foal in individual stalls, and mares and foals are turned out to a communal pasture about 7 days postpartum. The foal crop is doing well although virtually 100% of them experienced foal heat diarrhea (FHD) beginning during the second week of life. All foals recovered uneventfully within the next week or two. One colt (Ajax; born to a primiparous mare that had been purchased the previous year) developed watery diarrhea beginning at 31 days of age.

Clinical assessment

The foal now suckles less frequently than expected, and seems slightly lethargic. Ajax's physical exam reveals slight dehydration, and the perineum and hindquarters have been "scalded" by diarrhea. Watery, khaki-colored feces were passed in response to introduction of a rectal thermometer, and a sample was collected for diagnostic testing. The rectal temperature was 39.1 °C (102.4 °F).

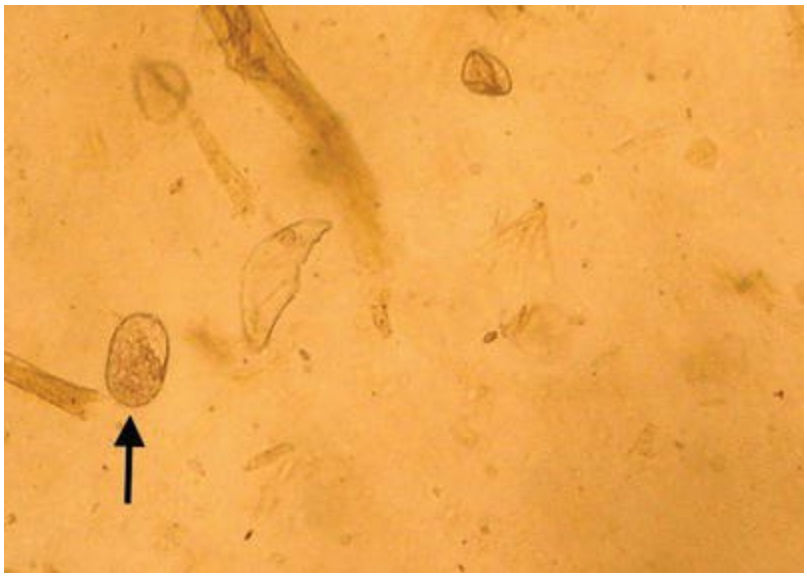
Diagnostics

Feces were submitted for a fecal flotation exam and bacterial culture.

Bacterial cultures revealed none of the typical foal pathogens (*Salmonella, Clostridium*), but the fecal exam (performed the same day) revealed high numbers of the objects depicted in Figure C9.1.

Questions

Figure C9.1 Parasite egg encountered in fecal samples (arrow).



1. The lab results were: "larvated strongyle eggs." Provide at least two reasons why one should discount this diagnosis as erroneous.

2. What is the correct diagnosis?

3. What treatment would you prescribe? Prognosis?

4. Should fecal samples from other, healthy foals be examined? Is rote treatment of the foal herd warranted without individual diagnostics?

5. Management recommendations for the mature members of the herd? For the stable facilities? For the pasture?

Answers

1. a. The minimum prepatent period for any equine strongylid infection is 5.5–6 weeks. This foal is too young to harbor a mature infection.

b. Strongyle eggs larvate only in the presence of aerobic conditions and a temperature greater than 7.5°C (45°F). This sample was collected fresh and processed promptly, so even if strongyle eggs had been present, they would not have had adequate time to larvate.

c. If the eggs were measured or examined closely, one might observe that they were smaller than the typical range for strongylid ova, were more round than elliptical, and had a thin shell.

2. *Strongyloides westeri* infection.

3. Treatment with either ivermectin (0.2 mg/kg) or oxibendazole (15 mg/kg). (Moxidectin is presumably effective, but is not labeled in the USA for use in foals less than 6 months of age.) The prognosis is excellent, and clinical signs normally disappear within 48 h.

4. The need for additional fecal testing is equivocal. If additional positive foals were identified, specific therapy is nevertheless unnecessary as long as they remain clinically normal. For the same reason, rote treatment is not advised, and any unnecessary use of anthelmintics can select for resistance in nontarget nematode populations (in this case, *Parascaris*).

5. *Strongyloides* is an unusual nematode in that infection is ultimately controlled 100% by acquired immunity. Immunity is fully effective by about 5 months of age, so mature animals are not threatened at all by this parasitism. In the past, it was a common management practice to deworm mares during the last month of gestation with a macrocyclic lactone anthelmintic to prevent lactogenic transmission of *Strongyloides* from the mare to her suckling foal. This measure is unwarranted, given the low incidence of *Strongyloides* disease in foals.

Sanitation of foaling facilities for the prevention of *Strongyloides* is not necessary, as long as mares and foals do not

reside therein for longer than 1 week. Infections can be transmitted by soiled bedding, particularly moist saw dust. This situation could arise if a 2 week or older foal (with a patent *S. westeri* infection) had contaminated a stall or pen, and other foals subsequently occupied the same area.

It is virtually impossible to eradicate *S. westeri* from pasture habitats. *Strongyloides* can pass several generations in the environment as free-living populations, even in the total absence of horses on the premise. Attempts at eradication would be fruitless.

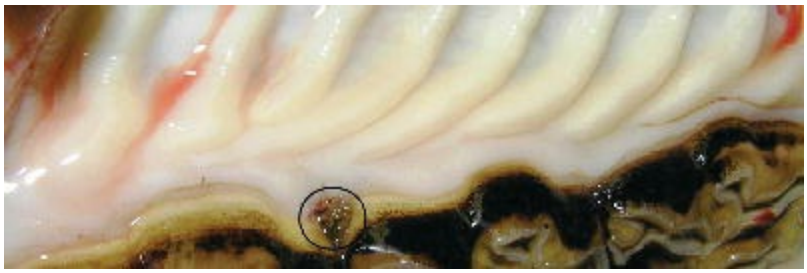
Case 10

Oral Lesion

History

A horse that has been salivating for several days is sedated and subjected to a thorough oral examination. The exam includes inspection of oral structures with a long dental mirror and a flash light. During this procedure, a worm-like appearance was noted and an image was captured with a flexible endoscope (Figure C10.1).

Figure C10.1 A larva (circled) is visible on the lingual border of a maxillary, interdental junction



Questions

1. Which parasite is this most likely to be?

2. What is the pathogenic impact of this parasite?

3. Which treatment recommendations would you give to the owner?

Answers

1. The localization and appearance is consistent with bot larvae of *Gasterophilus intestinalis*. As described in Chapter 2, first- and second-instar larvae reside in the oral cavity before they proceed to the stomach. Considering the extremely high prevalence of bots, this would not be an unusual finding in oral examinations conducted during the warmer months of the year.

2. Little is known about the pathogenic impact of bot larvae in the oral cavity, but they could possibly cause focal inflammation. It is now accepted that the life-cycle stages attached to the gastric mucosa only rarely cause clinical disease.

3. Ivermectin has demonstrated efficacy against oral stages of bot larvae. Moxidectin is also likely to be efficacious, but a definitive clinical demonstration has not been conducted. Horses probably profit very little from bot treatments. The major potential benefit may be fewer adult flies to lay eggs in the successive grazing season. But even then, one's bot control program is only as good as the neighbors'.

Case 11

Skin Lesion

History

A 4-year-old Arabian stallion from northern Indiana, U.S., was treated twice last summer for a small laceration on the medial, left front pastern. The laceration was not discovered until it was a few days old, and no attempt was made to suture it. Within a few weeks, the site had developed exuberant granulation tissue. The "proud flesh" was trimmed and the lesion treated by standard methods once in mid-August, and again in late September. The second treatment seemed more successful than the first, and the lesion shrank to minimal size (~4 mm × 14 mm) during winter. In early June, the owner calls to report that the lesion has returned and is larger than previously.

Clinical assessment

The skin lesion is ~3 cm × 5 cm, and extends ~5–7 mm above the surface of the surrounding skin. The lesion is ulcerated, and has a serosanguinous exudate. It is attracting flies and the stallion spends several minutes during each hour rubbing the lesion with its muzzle.

No other horses in the herd are affected. This stallion has received pyrantel tartrate on a daily basis for the past 2.5 years.

Diagnostics

McMaster egg count	75 strongylid EPG
CBC	WBCC 13.87×10^9 cells/L (slight leukocytosis)
Eosinophils	2.93×10^9 cells/L (absolute eosinophilia)

Questions (1)

1. What is the most likely parasitologic diagnosis?

2. How could you arrive at a definitive diagnosis?

3. What therapeutic plan would you pursue?

4. Should you examine fecal samples from other horses on the farm to determine whether they might be carriers of the offending organism?

Answers (1)

1. Cutaneous habronemiasis. Horses harbor three species of spirurid worms that reside as adults in the stomach. *Habronema muscae* is by far the most common. *H. majus* has never been very prevalent, and *Draschia megastoma* has become increasingly rare since the advent of macrocyclic lactone anthelmintics. These nematodes require dipteran intermediate hosts, which apparently deposit the larvae onto fresh wounds or mucocutaneous junctions, where they can invade the dermis and cause local pathology as described here.

2. The appearance of the lesion and accompanying exudates, pruritus, absolute eosinophilia, and regression of lesions during winter months are all typical for cutaneous habronemiasis. Regardless, a definitive diagnosis requires biopsy of the lesion and histopathologic demonstration of nematode larvae in the tissues.

3. The larvicidal properties of systemic macrocyclic lactone treatment are often sufficient to effect a clinical cure. Systemic corticosteroids have been used with success, and daily bandaging with some combination of antibiotics, corticosteroids, organophosphates, or DMSO are helpful. Large lesions might require surgical debulking in addition to medical therapy.

4. Additional fecal testing would be fruitless. The egg stages of *Habronema* and *Draschia* are quite small, and might not survive the flotation process. To eliminate other horses (albeit temporarily) as sources of spirurid larvae, the simplest approach would be to deworm them with ivermectin.

Alternate fate

You later discuss this case with a colleague, who mentions that she has been treating a similar horse for several months with little, if any, success.

Questions (2)

5. Is anthelmintic resistance a possibility?

6. Alternative recommendations for the problem case?

Answers (2)

5. Many practitioners report that some cutaneous habronemiasis lesions do not respond to anthelmintic therapy as predictably as they did 10 years ago.

> Although resistance to macrocyclic lactones has not been documented in spirurid nematodes of horses, it is biologically quite feasible. Gastric *Habronema* infections are fairly common, so this genus could be exposed to selection pressure when members of a herd are treated with ivermectin or moxidectin. Unfortunately, no antemortem tests are capable of demonstrating resistance in these worms. One would have to conduct a classic efficacy study involving a control group and postmortem worm counts.

6. A lack of response to macrocyclic lactones might require one to dust off the pharmacopeia of antiquity. In the good old days, topical or systemic organophosphates were used, with varying success, against these dermal infections (see Chapter 7).

Case 12

Legal Case

History

A 1-year-old pony was sold on October 1 in Denmark. The new owner decides to keep the pony in the stable of origin for another 6 months before she transfers it to her own facility on April 1. On April 4, the new arrival develops profuse, watery diarrhea and clear manifestations of colic, with cyanotic mucous membranes and a heart rate of 80–100 bpm. Despite intensive fluid therapy and colic management, the pony does not recover and is euthanatized. The attending veterinarian performed an autopsy and found that the cecal and ventral colonic mucosa were extremely edematous, and had numerous, pinpoint hemorrhagic lesions consistent with newly emerged cyathostomin larvae. The mucosal surfaces were hemorrhagic and inflamed, with areas of necrosis. Huge numbers of cyathostomin larvae and worms were found in the luminal contents. The veterinarian diagnosed this case as acute larval cyathostominosis. After consulting with her veterinarian, the owner decided to file a civil lawsuit against the seller of the horse, claiming that the pony had acquired the parasites before October 1 of the previous year.

The court calls you as an expert witness on equine parasite infections and proposes a number of specific questions. You are presented with the historical information shared previously; there is no mention of anthelmintic treatments.

Questions

1. Is it likely that the pony was already infected with strongyle parasites on October 1?

a. If yes, please explain how.

b. If no, please explain how the parasites could have been acquired between October 1 and April 1.

2. Would it have been possible to detect the parasite burden in question during a prepurchase examination in September? Could any diagnostic method have detected this condition prior to the onset of disease on April 4?

3. Could the disease occurring on April 4 have been prevented or mitigated if the pony had been treated with an anthelmintic after October 1?

4. What role could transfer to a different facility and change of housing and feed have played as a predisposing factor for larval cyathostominosis?

Answers

1. Yes, it is highly likely that the pony had acquired these strongyle parasites prior to October 1.

a. Strongyle parasites are ubiquitous, and the grazing season defines the time of transmission. In Denmark, the grazing season typically extends from May through October.

b. If the pony had access to pasture after October 1, some additional larvae could have been acquired. However, this remains unknown because no additional information regarding the length of the grazing season or contemporaneous anthelmintic treatments was supplied in the testimony.

2. Fecal egg counts do not reflect encysted parasite burdens and would have had no diagnostic value in the present case. Low plasma protein values could, in theory, support suspicion of a large, encysted cyathostomin burden, but no definitive tools exist for diagnosis of encysted cyathostomins.

3. No information was available regarding prior anthelmintic treatment of this pony. In general, anthelmintic treatments between October and March (i.e., while large numbers of larvae are encysted) would have comprised a risk factor for larval cyathostominosis. There is a distinct possibility that this clinical syndrome was initiated by an anthelmintic treatment just prior to transfer. Larvicidal treatment with moxidectin performed in the late summer or fall would likely have reduced the burden of encysted larvae and thereby the risk of larval cyathostominosis in the following spring.

4. The scientific literature has not identified stabling type and change of feed as risk factors for larval cyathostominosis. However, relocation is recognized as a potential stressor, as detailed in Chapter 4, and could pose a risk factor for the development of larval cyathostominosis.

Case 13

Repeated Egg Counts

History

The owner of a riding school contacts you in the spring for your advice regarding their parasite control program. The horses on this farm are all adults, ranging between 6 and 24 years of age, and several breeds are represented. Until recently, this farm had dewormed all horses at regular intervals year-round, but the owners had done some online research, and were interested in changing to a program based on fecal egg count results. They expressed some skepticism about egg counts, because they had also read that repeated counts from the same horse "could jump all over the place." As a mutual learning exercise, you offer to perform three repeated egg counts from each horse to illustrate the variability to the owner. The results are presented in Table C13.1.

Table C13.1 Results of three repeated strongyle egg counts from individual horses.

Horse	FEC1	FEC2	FEC3
A	60	20	20
B	0	0	0
C	200	260	260
D	540	420	400
E	0	20	20
F	0	0	0
G	760	1060	680
H	0	0	0
I	0	0	60
J	80	0	60
K	0	20	0
L	100	160	20
M	100	80	40
N	0	0	0
O	0	0	0
P	40	60	40
Q	0	0	0
R	0	0	0
S	40	60	40
T	0	0	0
U	0	0	0
V	0	20	20
W	0	40	20
X	60	20	0
Y	640	520	500
Z	1020	820	760
AA	0	0	0
BB	40	20	40
CC	20	0	20
DD	420	380	260
EE	620	500	600

Questions

1. The riding instructor is now even more skeptical about the reliability of fecal egg counts, and argues that it appears to be totally coincidental whether or not a certain horse should be treated. Interpret these results and explain your findings to the owner.

2. Based on these samples, recommend a complete, annual parasite control regimen for this farm.

Answers

1. The variability in egg counts is within the expected range (± 50%), and no horses exhibited extreme variability. The overall distribution of egg counts is typical for adult horses, and the majority are shedding ≤ 200 EPG. Only four of these horses (G, Y, Z, EE) would be classified as High Contaminators.

Although it may be a challenge to convince the riding instructor, a number of arguments should be made: (1) Egg counts should always be interpreted within broad ranges, and adult horses are likely to return to these levels after treatment. (2) The results have the expected variability, and there is good consistency between repeated counts. (3) If 200 EPG were chosen as the cutoff value for treatment, categorical recommendations (i.e., treatment vs. no treatment) would not have changed for any of these horses between sequential egg counts. (4) Although repeated egg counts can fluctuate just above or below the cutoff value, an adult horse is likely to tolerate a low worm burden without treatment. If the riding instructor is still skeptical, it is always possible to adjust the cutoff value.

2. The egg counts of this herd are not alarming. However, it should be remembered that riding schools often experience substantial turnover, so parasite levels can change quickly. As a basic foundation, this herd could receive one yearly treatment with a combination of praziquantel plus ivermectin or moxidectin to manage tapeworms and help prevent large strongyles. This treatment should be administered in the autumn. Additional treatments could be administered in the spring, based on fecal egg count results. A cutoff value in the range of 100–300 EPG should be identified, and horses exceeding this value should be treated with ivermectin. The six highest egg count horses should be chosen to perform a yearly fecal egg count reduction test to monitor for ivermectin resistance. To achieve greater reduction of the overall strongyle contamination for the herd, it would be beneficial to treat High Contaminators with moxidectin. For these adult horses, additional anthelmintic treatments (i.e., other than autumn and spring) are not likely to be necessary.

Case 14

Repeated Colic

History

Danish Warmblood, gelding, 11 years old, 471 kg.

This horse had recurrent episodes of mild colic and was hospitalized in September 2010. The formal diagnosis was sand impaction of the cecum, which was confirmed by imaging. A contemporary, quantitative fecal result was 0 strongylid EPG, and blood work was unremarkable. The horse was treated symptomatically with analgesics and IV fluids, and sent home after a few days with a daily *Psyllium* substitution to alleviate the sand impaction. In late January 2011, the horse had two episodes of mild colic and was treated by a local practitioner. After a third episode of colic within 5 days, the horse was hospitalized for further evaluation and treatment. At the time of admission, the horse had not received anthelmintic treatment for the past 8 months.

Presentation

The horse was depressed, with mildly to moderately painful colic. The heart rate, respiratory rate, and rectal temperature were within normal ranges. Feces were loose and the horse was mildly dehydrated. By rectal examination, a firm impaction could be palpated in the cecum. No sand was detected in the feces.

Laboratory findings

Eosinophils	$0.76 \times 10^3/mm^3$ (absolute eosinophilia)
McMaster	40 strongyle EPG and 280 *Anoplocephala* eggs/g. A modified McMaster for detecting tapeworm eggs revealed a total of 563 *Anoplocephala* eggs in 30 g of feces
Abdominal tap	35 g protein/L; leukocytes—20×10^9 cells/L (elevated); differential count—27.5% eosinophils, 21% neutrophils, 44% macrophages, and 7.5% lymphocytes

Questions

1. What diagnoses would you include in your differential list?

2. Which treatments would you recommend?

Outcome

The horse continued to colic despite various treatments. The owner could not afford further treatment and the horse was euthanatized on February 4, 2011.

Necropsy

More than 300 tapeworms were found loose in the cecal contents. At the ileocecal junction, the mucosa was irregular and had lymphoid elevations protruding into the lumen. All recovered parasites were identified as *Anoplocephala perfoliata* based on morphologic criteria. In addition, three specimens of *Setaria equina* were recovered from the abdominal cavity. (The latter were the likely cause of the observed eosinophil counts both in the blood and the abdominal cavity.)

3. The owner wants to know if the horse was already infected with the parasites at the time of the previous colic episode in September. What is your opinion?

Herd management

Due to the nature of the case, the farm owner wished to investigate the level of tapeworm infection in the remainder of the herd. Eight adult riding horses were resident on the farm, and both serum and fecal samples were collected for tapeworm analysis. The tapeworm serum optic density (OD) values are presented in Table C14.1.

Table C14.1 Serum ELISA results for horses in the herd.

Horse	OD
A	1.709
B	2.000
C	2.000
D	1.990
E	0.235
F	0.482
G	0.117
H	1.627

On the modified tapeworm McMaster, only horse “A” had a positive tapeworm fecal result.

4. What is your interpretation of these results? Are the surviving horses at risk of developing similar clinical conditions?

5. What treatment recommendations would you offer to the herd manager?

Answers

1. **a.** *Clinical diagnoses*:

Tapeworm infection with associated cecal impaction.

Peritonitis.

b. *Differential diagnoses*:

Cecal invagination or other mechanical obstruction requiring surgery.

Cyathostominosis.

2. The horse was treated with praziquantel/ivermectin.

Supportive therapy included intravenous fluids and analgesics: metamizole, butorphanol, and flunixin. Antibiotics: benzyl penicillin, gentamicin, and metronidazole.

3. Tapeworms may interfere with local alimentary motility, and could have contributed to the cecal impaction, and possibly have interfered with the passage of ingesta through the ileum as well. Adult tapeworms could have been established in this horse at the time of the first hospital admission in September. (Most cestode infections are acquired during the grazing months, and burdens peak in autumn and early winter.) The plain McMaster technique used for routine fecal egg counts is not particularly sensitive for detecting tapeworm eggs, so a cestode infection could have been missed on that occasion.

4. Considered *in toto*, the test results suggest that this herd is widely exposed to tapeworm infection. At the time of examination, however, only one horse had detectable egg-shedding. This suggests that several of the horses harbored tapeworms, but none had a worm burden approaching the magnitude of that in the fatal case. It was concluded in this particular case that other factors had rendered the horse more susceptible to tapeworms.

5. The remainder of the herd was treated with praziquantel before turnout in spring. It was recommended that the entire herd be treated each autumn with a combination drug containing a macrocyclic lactone and praziquantel. For strongyle control, it was recommended that fecal samples be collected in the spring for FECRT, and that selective therapy be implemented thereafter.

Case 15

Ivermectin Efficacy

History

A client asks you to evaluate the efficacy of ivermectin in a herd of Warmblood show jumpers. You perform pre- and post-treatment egg counts on nine adult horses in total. These horses were treated with ivermectin paste, and post-treatment samples were collected 14 days later. A McMaster method with a detection limit of 20 EPG was used for both examinations, and the respective strongylid egg counts are presented in Table C15.1.

Table C15.1 Pre- and post-treatment strongyle egg counts from a herd of horses treated with ivermectin.

Horse	Pre-treatment	Post-treatment
A	800	20
B	360	0
C	280	0
D	200	0
E	340	0
F	220	0
G	200	0
H	200	0
J	1320	1280

Questions

1. Calculate the fecal egg count reduction percentage for the herd.

2. What is your interpretation of the FECRT result?

3. What will you recommend to your client regarding future use of ivermectin in this herd?

Answers

1. With horse J included, the calculated FECR is 88.9%. If "J" were excluded from the data set, however, the FECR would be 99.7%.

2. Horse J is a clear outlier, and should not be included in the FECR calculations. In all likelihood, "J" was either inadvertently skipped during treatment, or perhaps it managed to reject its dose of paste unnoticed. Supporting this conclusion is the observation that none of the other horses exhibited signs of reduced efficacy. However, these results

should be interpreted with caution, because all of the horses with 100% FECRs had low to moderate pre-treatment egg counts. A slight decrease in FECR therefore might not be detected because the detection limit of the McMaster was moderate (20 EPG). It is also worth noting that the two horses with positive, post-treatment egg counts also had the highest pre-treatment egg counts. See Chapter 9 for a further discussion of interpreting FECRT.

Viewed comprehensively, this FECRT exercise detected no evidence of ivermectin resistance, but a more reliable estimate of IVM efficacy could be achieved by (a) including more horses with higher egg counts, or (b) using an egg-count technique with a lower detection limit, such as the Flotac, Fecpak, Stoll, or Wisconsin (see Chapter 9).

3. The client should be advised to re-treat horse J (meticulously) with ivermectin and submit another post-treatment sample for evaluation of efficacy. Because this horse appears to be a high shedder, moxidectin might be a better drug choice. In general, avermectins and milbemycins appear to maintain good efficacy in this herd, although future reevaluations are warranted on an annual basis.

Case 16

Ten Commandments

History

In 2009, one of the authors presented the "Ten Commandments of Equine Parasite Control" at an international veterinary conference. The commandments are presented below:

1. Don't use an anthelmintic without knowing its efficacy against the intended parasite population.

2. Don't treat at frequent fixed intervals year-round.

3. Don't rotate blindly between anthelmintic drugs.

4. Don't treat adult horses during the season when environmental translation is minimal (unless there is a clear clinical indication to do so).

5. Don't treat the entire herd just prior to a move to clean pasture.

6. Don't treat pregnant mares just prior to foaling.

7. Don't treat at the first frost.

8. Don't intentionally under-dose any anthelmintic treatment.

9. Don't use anthelmintic formulations that are not labeled for horses, or administer them by a route that is inconsistent with label directions.

10. Don't use one standard treatment program for all horses in a stable.

Question

Explain the rationale for each of these "don'ts".

Answers

1. The prevalence and intensity of anthelmintic resistance have become so great that none of the anthelmintic drugs can be assumed to have 100% efficacy in every circumstance. The absolute starting point for all treatment regimens is to routinely evaluate the efficacy of available drugs.

2. Transmission of parasites is a biological system, and its intensity fluctuates with the seasons, the susceptibility of host populations, and innumerable other details. Although it may be easy to remember, it is totally illogical to implement parasite control as a regularly scheduled transaction, like a mortgage payment.

3. Anthelmintic drugs differ in their antiparasitic spectra, and one drug cannot be substituted blindly by a different product. The choice of drug depends on which parasite species are present, and the goal of the treatment. Contrary to conventional wisdom, there is no scientific proof that drug rotation delays the development of resistance.

4. If parasite transmission is controlled during the seasons of active translation, treatments outside this interval are

unnecessary. In nearly every climate, there are substantial intervals during the year when environmental conditions are unfavorable for parasite transmission, and anthelmintic treatments at those times are potentially harmful. Strongyles are the major parasites of adult horses, and they follow these rules very closely.

5. The traditional "treat-and-move" strategy is now discouraged because it contradicts current knowledge about the importance of parasite *refugia*. Clean pastures harbor minimal *refugia*, and the first eggs to contaminate this new venue will all be produced by worms that survived the recent deworming. This practice introduces strong selection pressure for the development of anthelmintic resistance.

6. The sole reason for treating pregnant mares was to prevent the transmammary transmission of *Strongyloides westeri* to foals. However, this parasite has become uncommon, it is not a serious pathogen, and foals of treated mares can still acquire infections from free-living populations on the pasture.

7. Although butterflies and dandelions disappear in winter, frost does not kill strongylid L_3s or ascarid eggs. As outlined in answer #4, winter treatments in a northern temperate climate are unnecessary, and could even introduce some risk of triggering larval cyathostominosis.

8. Under-dosing has been identified as a definite risk factor for the development of anthelmintic resistance. Pharmaceutical companies and the FDA take considerable pains to ensure that the label dosage of any product is both safe and effective. Under-dosing carries no assurance of efficacy, and its intentional implementation by a medical professional is irresponsible.

9. The pharmacokinetics and metabolism of drug formulations intended for administration to other animal species, or via other routes of entry, are generally unknown and hence accurate dosages cannot be extrapolated. This practice introduces considerable risk of under-dosing, and the possibility of illegal drug resides when employed for food animals.

10. Individual horses within a herd differ greatly in the numbers of worms they harbor, the degree to which they contaminate the environment, and their susceptibility to reinfection. In addition, juvenile horses might harbor *Parascaris* equorum, which requires separate treatment considerations, and a horse shedding very high numbers of strongyle eggs should be treated differently than a consistent low contaminator. Treatment needs must be tailored to the individual.

Case 17

Ivermectin Egg Reappearance

History

You have been using ivermectin almost exclusively for a decade or more in a small string of Quarter Horse brood mares that you own in partnership with a good friend. You are both curious about the status of ivermectin in this herd because you heard recently in an online webinar that a shorter egg reappearance period (ERP) after ivermectin treatment could be interpreted as evidence of developing resistance to the drug. You both decide to evaluate the efficacy of ivermectin in this herd, and to measure the ERP in a systematic fashion.

Nine mares with positive egg counts are selected and dewormed with ivermectin paste. During weeks 2 through 8 post-treatment, fecal samples are collected for quantitative analysis. The FEC results are presented in Table C17.1.

Questions

1. Calculate the weekly fecal egg count reductions (FECR) for this herd.
2. What is your interpretation regarding the current status of ivermectin efficacy in this group of mares?
3. What is your recommendation for future use of ivermectin?

Table C17.1 Strongyle egg counts of fecal samples collected prior to and weekly after ivermectin treatment (McMaster technique with a sensitivity of 25 EPG).

Pre-treatment	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
225	0	0	0	0	25	25	25
875	0	0	25	50	25	100	175
700	0	0	0	0	50	150	250
200	0	0	0	0	0	0	25
650	0	0	0	25	75	50	100
975	0	0	0	25	50	200	150
400	0	0	0	0	0	25	0
350	0	0	0	0	25	50	100
1025	0	25	0	75	125	250	250

Answers

1. The FECR results are presented in Table C17.2.
2. The mean FECR for the group does not fall below an 85% cutoff (see Chapter 9) until week 8. The result for week 7

could be considered border line. Overall, this herd exhibits an ERP of 7–8 weeks for ivermectin, which is considered satisfactory. This collection of data indicates no signs of ivermectin resistance.

3. The client can keep using ivermectin for strongyle treatment. If he is motivated for monitoring the ivermectin efficacy in coming seasons, you could suggest performing the post-treatment egg counts one time in weeks 5 or 6.

Table C17.2 Calculated, weekly fecal egg count reductions (FECR) for individual horses after ivermectin treatment. Mean FECRs are presented below each column.

Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
100	100	100	100	88.8	88.8	88.8
100	100	97.1	94.3	97.1	88.6	80.0
100	100	100	100	92.9	78.6	64.3
100	100	100	100	100	100	87.5
100	100	100	96.1	88.5	92.3	84.6
100	100	100	97.4	94.9	79.5	84.6
100	100	100	100	100	93.8	100
100	100	100	100	92.9	85.7	71.4
100	97.5	100	92.7	87.8	75.6	75.6
100	99.7	99.7	97.8	93.6	87.0	81.9

Case 18

Name that Worm

History

While evacuating the rectum of a 7-year-old Standardbred mare in preparation for reproductive ultrasound, the vet noticed one, fairly large (~3.5 cm), cream-colored nematode that was removed with the feces (Figure C18.1). After concluding the procedure, two similar worms were found adhering to his OB sleeve.

The mare had not been dewormed for 6 months, and had exhibited no abnormal clinical signs. The owner requested a diagnosis, and wanted to know about therapeutic and management recommendations.

Figure C18.1 Nematode removed with feces

Questions

1. What is the parasitologic diagnosis?

2. Should other horses in the herd be screened?

3. Which anthelmintic(s) would you recommend?

Answers

1. Pinworm infection (*Oxyuris equi*). The observed specimens were likely adult females; males are much smaller and generally less numerous. These worms normally inhabit the rectum and distal descending colon, and are commonly seen on freshly passed feces. The only unusual feature of this clinical presentation was that the infection occurred in a mature horse. Historically, most pinworm infections have been observed in juvenile horses.

2. Diagnostic screening would not provide many definitive answers. If one horse is infected, the remainder of the herd has probably been exposed. Pinworm infections are more likely in immature horses than in mature stock, but this historical pattern seems to be changing on many farms. Pinworms do not lay eggs in feces, so screening by flotation or quantitative procedures would be fairly useless. Pinworm eggs are best detected by the Scotch tape technique, or by perianal scraping (see Chapter 8).

3. Any broad spectrum equine anthelmintic, by definition, should deliver >90% efficacy against adult *Oxyuris*. Thus, pinworm populations should be removed by label dosages of macrocyclic lactones, pyrimidine products, or benzimidazoles. In recent years, however, many practitioners have reported failures of macrocyclic lactones (even repeated treatments at elevated dosages) to remove pinworm infections of mature horses. A recent study with ivermectin (0.2 mg/kg) and pyrantel pamoate (13.2 mg/kg) found no evidence of resistance in the pinworm strains evaluated.

Stall hygiene would have limited benefit for preventing infections. Anthelmintic treatment could logically be restricted to horses with clinical signs, and frequent bathing of the perianal region might help to relieve pruritus.

Case 19

Parasite Control for Yearlings

History

A local trainer contacts you in late winter with questions about deworming her charges prior to the approaching grazing season. All of her horses are youngsters, ranging from weanlings to a 3-year-old. The results of recent quantitative fecal exams are presented in Table C19.1.

Questions

1. Based on the egg counts, would you treat any of these horses?

a. If so, which ones?

2. List the anthelmintics that you would consider using.

a. Provide the rationale for your recommendations.

Table C19.1 Ascarid and strongylid fecal egg counts (EPG) of young horses in training.

Horse	Age*	Strongyle	Ascarid
A	1.5	350	0
B	1.5	500	0
C	1.5	500	0
D	0.5	300	0
E	0.5	150	150
F	0.5	0	100
G	1	200	50
H	3	0	0

*Expressed in years.

Answers

1. None of the egg counts are alarmingly high. In fact, the strongyle egg counts are relatively low, considering the age of the hosts. But, it should be remembered that egg counts are often lower during winter months. The presence of ascarid eggs in horses of this age group is not unexpected. Anthelmintic treatments could be considered, but it primarily depends on when

these horses were last treated.

a. A major consideration regarding treatment at this time is the presence of ascarids in three foals (E-F-G), especially the possibility of small intestinal impaction. If these horses had been treated for strongyles in the fall, and they currently appear healthy, strongyle treatments could wait until spring, when active translation can occur on pasture.

2. For ascarid treatment, a benzimidazole is recommended. If the trainer elects to treat for strongyles, ivermectin and/or moxidectin would be a rational choice, but pyrantel could also be used if known to be efficacious.

a. The risk of small intestinal impaction appears to be considerably less with benzimidazole treatments compared to drugs with paralytic modes of action (see Chapter 7). In healthy horses, the primary reason for a strongyle treatment is to decrease egg shedding. Benzimidazole resistance is extremely prevalent in cyathostomins, and cannot be expected to work unless a fecal egg count reduction test has indicated otherwise. Pyrantel could work on many farms, but again, the local efficacy needs to be verified. Moxidectin is particularly useful for larvicidal therapy, treatment of clinical cases of parasitic disease, and treatment of extreme high contaminators. Ivermectin/moxidectin resistance may be an emerging problem among cyathostomins, so it is recommended to monitor their efficacy on a routine basis.

Case 20

Reaction to Treatment

History

During May in Louisiana, U.S., a client deworms her 10-year-old Paint gelding with generic ivermectin in preparation for pending competitions. Two days later, she notices some significant swelling along the ventral midline, and the horse is rubbing its chest on the stall door and its withers against trees in the pasture. None of her other horses are exhibiting similar behavior.

Clinical presentation

Upon closer examination, the dependent edema involves areas of discrete hair loss with slight crusting along the ventral midline. Multiple, small lesions are also noted on the face, neck, and withers, but these are limited to slight hair loss and focal accumulations of gray scales. A cursory exam reveals that several pasture mates have similar lesions, although none are exhibiting severe pruritus.

The show season for this horse has abruptly ended, and the owner wants to know if the manufacturer has any legal liability for this obvious allergic reaction to ivermectin.

Questions

1. Can you give a plausible parasitological explanation for the observed reactions?

2. How could you achieve a definitive diagnosis?

3. What are your treatment and future management recommendations?

Answers

1. The lesions in all the pastured horses are consistent with *Onchocerca* spp. infection. The focal host reactions develop in response to microfilarial stages in the dermis. Microfilariae are killed by macrocyclic lactones, and pruritus, edema, and local inflammation of the dermis are common reactions to dying worms, not a reaction to the anthelmintic itself. All horses on the premises should be similarly exposed to *Onchocerca* infection, which is transmitted by the bite of arthropod vectors (*Culicoides* spp.).

2. Diagnosis can be confirmed by demonstrating motile microfilariae in skin biopsies (see Chapter 8). Because microfilariae in the treated horse are probably dead, diagnostic efforts would be more productive if skin biopsies from one of the untreated pasture mates were examined.

3. Macrocyclic lactone treatment is usually sufficient to kill the microfilarial stages and alleviate the skin lesions. The adult

worms, which reside in deep connective tissues, are not killed by ivermectin, but apparently are rendered infertile for intervals of several months. Microfilarial production eventually resumes, so intermittent macrolactone treatment may be required to prevent recurring skin lesions. Measures intended to limit *Culicoides* exposure (e.g., nocturnal stabling, window screens, overhead fans) are useful adjuncts for preventing infection.

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