A Practical Method of Identification of the North American Cyathostomes (Small Strongyles) in Equids in Kentucky

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Contents

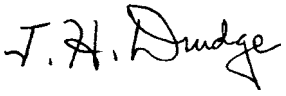
Foreword

Small strongyles are the most common of the internal parasites of the equid. Infections of these nematode parasites occur in all ages from very young foals to the aged. The small strongyle grouping is uniquely large, as more than 50 species have been described. They live in the cecum and large colon of equids, and very large numbers of adult worms and developing larvae tend to accumulate. Massive infections may be lethal, particularly in young equids (weanlings and yearlings). The resultant clinical entity, known as *larval cyathostomiasis*, has emerged in the past 15 to 20 years as one of the most important diseases of the horse.

Differentiation or identification among the small strongyle complex is particularly challenging. It requires inspection of each worm under a compound microscope. Of the 50 species of small strongyles described, 33 species have been found in Kentucky equids, largely due to the diligent efforts of the author of this treatise on small strongyles.

Sharon Tolliver is a research specialist in the Department of Veterinary Science. She joined the department's parasitology group in 1965 and earned the B.S. in Agriculture in 1968 and the M.S. in Veterinary Science in 1991. Ms. Tolliver's master's degree research dealt with drug-resistant small strongyles in Kentucky equids.

Years of dedicated effort in determination of species of small strongyles have resulted in this unique proficiency possessed by Ms. Tolliver, an authority on small strongyle identification. Her scheme for differentiation of this fascinating group of nematodes is revealed herein. The approach is not an orthodox one, but it has proven to be practical, efficient, and effective. The material should be helpful to others challenged to identify members of this complex group of nematodes.



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Acknowledgments

This monograph is dedicated to classical parasitologists worldwide, especially those whose research interest has concentrated on the cyathostomes. I would like to thank some of our colleagues, with whom we have worked closely for many years, for their collaboration and friendship. They are Tom Klei, Ph.D., Melanie Chapman, B.S. (Louisiana State University); Rosina "Tammi" Krecek, Ph.D., Susan Milne, National Diploma (University of Pretoria, the Republic of South Africa); Joe DiPietro, D.V.M., M.S. (formerly a professor at the University of Illinois and now dean of the College of Veterinary Medicine, University of Florida); Ray Kaplan, D.V.M., Ph.D., Dave Goodman, M.S. (University of Georgia); and especially Craig R. Reinemeyer, D.V.M., Ph.D. (formerly a professor at the University of Tennessee and now a private clinician in Knoxville), whose encouragement led to the publication of this monograph.

I was so fortunate to have J. H. Drudge, D.V.M., Sc.D., as my mentor and guide while I was learning to identify these parasites because he knew most of the more common ones. Not only was he the senior parasitologist at that time, he was also the chairman of the Department of Veterinary Science. Busy as he was, he never lost patience with me and was an avid instructor. Now retired and a professor emeritus, he is still very active and regularly comes to the parasitology laboratory to edit manuscripts and discuss the research.

Particular appreciation is expressed to Peter Timoney, M.V.B., Ph.D., chair of the Department of Veterinary Science and director of the Gluck Equine Research Center, University of Kentucky, for his enthusiastic support of this project.

There are no superlatives to describe the contribution of E. T. Lyons, M.S., Ph.D., professor of parasitology, to the preparation of this monograph. He worked tirelessly after hours and on weekends for nearly a year, taking hundreds of pictures of the cyathostomes to obtain the best possible representative of each species. In addition to the photography, his input and suggestions regarding layout and method of illustrating the size of the worms and his attention to every detail were invaluable to this publication. He has been my mentor and friend for more than 35 years, and the enthusiasm, hard work, and talent he contributed to this project are indicative of why he is one of the best known and most respected classical parasitologists in the world.

I also want to express appreciation to Tetiana Kuzmina, Ph.D., Department of Parasitology Institute of Zoology, National Academy of Sciences of Ukraine, Kiev, Ukraine, for identification, description, and photographs of four species new or updated with this revision.

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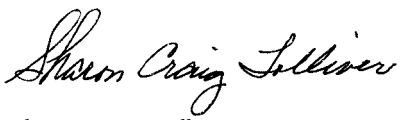

Sharon Craig Tolliver

Introduction

At the present time, there are 51 recognized species of cyathostomes (small strongyles) in the world (Lichtenfels et al., 1998) in equids. Until Lichtenfels in 1975 and Lichtenfels et al. in 1998 reclassified the cyathostomes, we had reported that 33 species were found in Kentucky. Six of these species have now been moved to the large strongyle category, and one species has been deleted from the strongyle category altogether. However, we have always regarded those species that do not migrate outside the gastrointestinal tract as the small strongyles, and only the three species (*Strongylus vulgaris, Strongylus edentatus,* and *Strongylus equinus*) with parenteral migration, e.g., into the cranial mesenteric artery, liver, or pancreas, as the large strongyles. So, for the purpose of this monograph, all 33 Kentucky species we learned to identify as small strongyles will be included.

The Challenge of Identification

Because there are so many species, those interested in learning them may be overwhelmed and defeated before they start. Also, very seldom is someone present who can confirm or contradict the identification of a species. Moreover, the available publications describing these parasites can be very confusing to the beginner because most of the details concentrate on a series of measurements (tails, vulvas, dorsal rays, etc., in millimeters and microns). These descriptions may work for taxonomists, but they are of very little use to the ordinary technician or parasitologist who needs to recognize these parasites on sight.

We learned to speciate the cyathostomes by "digging them out." We did not have access to the wonderful photographs and drawings available in Bowman (1995), Georgi (1980), or Lichtenfels (1975). Our colleague, Dr. J. H. Drudge, in the 1950s, obtained microfilm copies of most of the original descriptions of small strongyles from scientific journals. He made positive prints on photographic paper of pages with drawings and plates; he also had the descriptions typed. This material is in our parasitology collection of literature. Other helpful references we used were Looss (1902) and Theiler (1923).

When we first began the identification of these parasites from drug tests, we set up a standard protocol that we have followed for more than 30 years. We decided to identify at least 1,000 worms per animal or the total recovered, whichever came first, from the population of worms in each horse. It soon became apparent that we were looking at only about 10 species again and again. This has remained consistent since the outset. About 95 percent of the small strongyle population in a horse in Kentucky typically is comprised of the same 10 species: *Coronocyclus coronatus, Cyathostomum catinatum, Cylicocyclus insigne, Cylicocyclus leptostomus, Cylicocyclus nassatus, Cylicostephanus calicatus, Cylicostephanus goldi, Cylicostephanus longibursatus, Cylicostephanus minutus,* and *Poteriostomum imparidentatum.* Of these species, three (*Cyathostomum catinatum, Cylicocyclus nassatus,* and *Cylicostephanus longibursatus*) comprise

2012 Revision

Revision of this monograph includes addition of three species *(Cylicostephanus bidentatus, Cylicostephanus hybridus,* and *Cylicocyclus ashworthi)* not previously reported in Kentucky. Also, a redescription and new photographs of *Cylicostephanus asymetricus* are included; note that in the original monograph the photographs and description were actually those of *C. bidentatus.*

about 80 percent of that total. Essentially, these same species and percentages were found in the United Kingdom (Ogbourne, 1978) and elsewhere in the United States (Reinemeyer, 1986).

The above 10 species are known to show resistance (seven in Kentucky) to every parasiticide on the market worldwide except the macrocyclic lactones. With the increase in reported cases of *larval cyathostomiasis* (Lyons et al., 2000; Reinemeyer, 1986), these parasites, formerly regarded as relatively benign, can become a dangerous problem for horse owners because it is just a matter of time before these tenacious little invaders "figure out" the macrocyclic lactones just as they did the benzimidazoles.

The Need for Identification

Now that veterinarians and researchers are beginning to accept the pathological consequences that can be caused by this group of parasites, more and more researchers want to learn to identify them. Fortunately, for those just learning, the reality is that they will probably see fewer than one-third of the 33 species. Additionally, these species are the most prevalent and in the greatest numbers; consequently, they are the most dangerous to equids. Once a person is familiar with these, a rare species will "stick out like a sore thumb." The fact that a species is so different will be noted and its characteristics easily remembered.

The species that occupy <1% of the total population in the lumen of a horse are very difficult to recover, and some technicians may never see some of them. We have done so many critical tests that we have been fortunate enough to recover all of them and even one (*Cyathostomum alveatum*) that had never been reported in the United States (Tolliver et al., 1985). Through this research, we discovered that even though the various species of cyathostomes can probably survive in almost any equid, some prefer donkeys or zebras to horses.

Probably the reason some species are not seen is that researchers are not looking in the right place. To recover a lot of specimens of *Cyathostomum alveatum, Cyathostomum tetracanthum, Cylicocyclus auriculatus, Cylicocyclus ultrajectinus, Poteriostomum ratzii,* and *Triodontophorus tenuicollis,* researchers need to find a donkey that has not been dewormed recently or at all. We found these species in abundance in a group of donkeys purchased for a lungworm experiment.

Mastering the identification of the small strongyles is a long-term commitment, and one has to look at thousands of specimens over and over until their features become so familiar that just a glance will reveal their identity. I have probably looked at more than 500,000 specimens during the past 30-plus years, applying what I call the "old country doctor method"; that is, I could identify each species because I had seen it before. Even after learning to identify these parasites, one needs to keep up this skill by continuing to "practice" with them.

A Unique Method of Identification

Parasites are like people; they come in all sizes, even if they are the same species; some eat more or are older/younger, sick/healthy, etc. My method does not involve measuring lengths and widths because this is time-consuming and inefficient. Also, I learned to identify these parasites from those recovered from the horses' feces after deworming; they were dead and mangled from the effect of the parasiticides. Therefore, identification was possible only by recognizing the individual tell-tale characteristics exhibited by each species.

Each person needs to pinpoint some distinguishing feature of a species that will identify it every time. It is not important to know the taxonomic term for a certain element; the objective is the correct identification of the species, whatever the method. For example, one person may see features on a worm that look like "a pair of giant ears"—even "Mickey Mouse ears," while that same feature may remind someone else of old-time chicken-feed sacks tied at the corners. This variation in the way a particular feature is described only illustrates that those interested in learning to identify these parasites *must* hone in on something about each species that will identify it *for them*. It does not matter what feature works as long as the specimen is identified correctly.

The trick to identifying any species is first to eliminate what it is not. Identifying species by specific measurements does not work, but one does have to be aware that each of the 33 species is either small, medium, or large. Once that has been established, just a glance at a specimen will eliminate two of the three groups; e.g., *Oesophagodontus robustus* is the largest species (19 to 22 mm), and *Cylicostephanus minutus* is the smallest (4 to 5.5 mm). So, those two species could never be confused with each other. Most of the species (19) are small; I consider six to be medium-sized species, and eight large. When I look at a specimen, I gauge it in terms of too big, small, thin, long, stout, bursa too short, female tail too tapered, etc., rather than through direct measurement.

Identification of the small strongyles is not that difficult if approached in this logical manner. The species is either small, medium, or large. The buccal capsule (mouth) is either round, rectangular, or square. Each has some characteristic, usually the shape of the buccal capsule walls, that is significant only to it. However, some have other distinguishing characteristics; for example: (1) leaf elements of the internal or external crowns that are prominent, (2) some males have extra long bursas, (3) females have tails that are either tapered to a point, end bluntly in a stub, or turn foot-like, and (4) one has a very long and straight esophagus. Species such as *Craterostomum acuticaudatum*, *Cyathostomum tetracanthum*, *Cylicocyclus auriculatus, Gyalocephalus capitatus*, *Oesophagodontus robustus*, *Petrovinema poculatus*, and the *Triodontophorus* spp. are so unusual and distinctive that anyone should be able to recognize them at once.

The most useful characteristic in the identification of the small strongyles is the buccal capsule—not only its shape but, most important, its walls. Each looks different, and I learned to associate the way these walls looked with everyday objects. Also, after a while, the size and shape of the male (bursa) and female tails became familiar and provided an additional confirmation of a worm's identity.

Information on each species of Kentucky cyathostomes is presented in several ways: photographs showing different views of the head and tail, a descriptive narrative, and an abbreviated checklist. Repetition of certain features is intentional to emphasize those characteristics that have been most useful to me in the correct identification of each member of this complex group of parasites.

Protocol

Until a species can be identified, it needs to be fixed in a preservative for a period of time, at least a few hours. We routinely fix our specimens in a mixture of alcohol/formaldehyde/ glycerine (AFG); this makes them clear much faster than fresh ones. Next, we place them in a clearing solution so that the internal structures are visible. We have always used beechwood creosote as our clearing agent; some others use lactophenol or glycerine. Beechwood is very pungent and quite caustic. It will not burn the skin, but the odor permeates clothing and working facilities and should be used in a spacious area and as quickly as possible. However, we have found it to be the most effective medium because it clears the small worms almost immediately; it takes a little longer for the larger ones.

I view all the species at 10X. My technique is to place three drops of beechwood on a slide and put four worms (if they are small) in each drop, line them up head-to-head, and top them with three coverslips—12 worms/slide. This allows me to cross the slide quickly, identifying 12 worms in about 30 seconds. Because we have always been interested in the effect of the parasiticides on reproduction, I note the sex of each species and whether the females have eggs. I simultaneously learned to identify the worms from both the anterior and posterior views. Now, I can usually identify a worm by its posterior before I ever look at its anterior end.

Most worms die "on their backs" because the majority of specimens present a dorsoventral view under the coverslip. Sometimes the larger ones want to turn on their "side," and the view is lateral. If a worm does not show its "face," a nudge with a probe or a pair of tweezers against the side of the coverslip will cause the specimen to roll, and then identification is easier. Occasionally, a very large species will not cooperate because the worm is bent or distorted, and it becomes necessary to sever its head so it can be rolled; however, this is very infrequent. Often, debris in the buccal capsule obscures internal structures.

Most of the photomicrographs were taken with a camera mounted on a Zeiss compound microscope equipped with a Nomarski differential interference contrast attachment at the objective powers of 2.5X, 6.3X, 16X, or 40X. For the photomicrographs, scale bars designate the size of the specimens. However, for a few, only the magnification is given because these were taken many years ago through a microscope for which the information on actual size has been lost.

Keys to the Cyathostomes (Small Strongyles)

The following are the 33 species and the "scheme" by which I learned to identify them. The genera are in alphabetical order with the species also alphabetized; however, a few will be out of order for reasons explained. Any of the species can be found anywhere in the gastrointestinal tract, but each species prefers its own niche (Mfitilodze and Hutchinson, 1985) designated here as cecum (CEC), ventral colon (VC), or dorsal colon (DC). However, sometimes it is difficult to pinpoint the preference of some of the very rare species because their recovery is so infrequent; then it is only possible to note where they were found in each case.