

Some Historic Aspects of Small Strongyles and Ascarids in Equids Featuring Drug Resistance with Notes on Ovids

EMPHASIS ON RESEARCH AT THE UNIVERSITY OF KENTUCKY

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Some Very Early Records of Horse Parasites

Internal parasites of horses were observed by humans hundreds of years ago. Morgan and Hawkins (1949) in their book state that some larger parasitic worms were studied even by ancient scientists “as a possible cause of disease.” They mention that Hippocrates (430 BC) noticed the pinworm (*Oxyuris equi*) in horses; Columella (100 AD) first observed an ascarid from a calf; and Vegetius (400 AD) observed an ascarid from a horse. LeRoux (1924) states that one of the earliest descriptions of a species of small strongyles, which he believed to be *Cylicostomum longibursatum*, was by O. F. Muller in 1780. Looss (1901) in his classical monograph described several distinct species of strongyles in horses. Also, he mentioned that Goeze (1782) used the term “pallisadenwurm” to describe the armature of the head, or “corona radiata,” which is a morphologic characteristic of strongyles in horses. Poinar (1983) states that Dubinina (1973) found specimens of *Alfortia edentatus* (now *Strongylus edentatus*) in the intestine of an Upper Pleistocene horse. When compared with present-day species, it had not changed in the past 33,000 years.

Small Strongyles and Ascarids—General

Horses may harbor more than 100 species of internal parasites. About one-half of these are in the group called small strongyles. Some of the genera from this group are now taxonomically placed in the large strongyle group. The remaining so-called “small strongyle” species are called “cyathostomes” or “cyathostomins” by some. However, in this paper, the historic name “small strongyles” is used to include all horse strongyle species except those in the “large strongyle” genus *Strongylus*. Identification of small strongyles is aided in publications by Georgi and Georgi (1990), Lichtenfels (1975), Lichtenfels et al. (2008), and Tolliver (2000). Small strongyles are commonly found in the large intestine of horses of all ages. Ascarids (*Parascaris equorum*) are the large round worms typically found in the small intestine of young horses.

Purpose of This Publication

The present bulletin focuses mainly on drug-resistant species (small strongyles and ascarids) of internal parasites of the horse with emphasis on historic research by Hal Drudge, DVM, ScD, alone and with colleagues at the University of Kentucky (UK). Some discussion is presented also of research at UK on the sheep “barber pole” stomach worm (*Haemonchus contortus*) which has a historic role in drug resistance. Drudge’s foresight and outstanding personal observations in some of the pioneer parasitologic research are recorded here. Drudge made huge contributions to the knowledge of veterinary parasitology. He came to UK in July 1951 after a short stay at Mississippi State University after earning his ScD degree at Johns Hopkins University in 1950. The focus here is on his extensive research on one of the major aspects of parasite control, drug-resistant nematodes in horses and sheep. It is not surprising that early in his professional career, Drudge had an interest in sheep parasites. While still in veterinary school at Michigan State University, he was a co-author on three papers on internal parasites of sheep (Hawkins et al., 1944a, b, c). It should be mentioned that some other scientists (W. W. Dimock, M. E. Hansen, and A. C. Todd) in the Department of Animal Pathology (now the Department of Veterinary Science) at UK had done excellent research on internal parasites of domestic animals, particularly horses, before Drudge arrived. Thus, he was effectively able to “hit the ground running” in his research.

Parasite Control in Horses and Sheep

Over the centuries, parasitocidal attributes have been reported anecdotally for numerous procedures and substances for treatment of parasites in horses (Poynter, 1959). An early scientific study on chemical control of horse parasites, showing efficacy especially against strongyles, was for oil of chenopodium (Hall et al., 1918). However, the drug had rather drastic side effects in horses. Later, more broad-spectrum and less toxic compounds were developed.

Phenothiazine—General

Research studies on phenothiazine (PTZ), beginning in the 1940s, indicated that it initially had great activity against several species of nematodes in horses and other animals compared to other compounds and that it was relatively nontoxic to the hosts. Also, it was the first compound for which drug-resistant nematodes later were found. Therefore, it seems appropriate to relay several aspects about this drug.

Taylor and Sanderson (1940), in the “General Usefulness” section of their paper on PTZ, discuss the advantages of this drug for treatment of internal parasites in sheep. Then they state, “Its greatest use may, however, prove to be in the treatment of strongylosis in equines, where it can be relied upon to exert a 100 percent efficiency. If the safety of this treatment is also taken into consideration there seems to be every reason for its frequent and regular employment in horses and it is by no means too extravagant to suppose that by regular dosings every two or three weeks it may prove possible to eradicate the infestation from certain environments, a procedure which could not previously have been contemplated with high hope of success.” Yet, an old adage warns that we should be wary of something that seems too good to be true. This saying turned out to be appropriate for this chemical and many succeeding parasiticides because of the later development of drug resistance of parasites.

Phenothiazine Resistance of *Haemonchus contortus* in Sheep

Drudge, with *H. contortus* in sheep (Drudge et al., 1954), was the first person to publish on experiments proving drug resistance of nematodes. In that publication, he acknowledges that Schwartz, in personal communication (1953), knew of field reports of resistance of *H. contortus* to PTZ.

Drudge documented drug resistance of *H. contortus* to PTZ by field tests and through information from necropsies of dead lambs brought to the postmortem room at the Animal Pathology building on the UK campus by a well-known and highly respected local sheep man (Drudge, personal communication). The pathologist who did the necropsies told Drudge about the unusual numbers of deaths of lambs from haemonchosis after PTZ treatment on this one farm. Drudge knew that the farm’s owner had a routine parasite control program. The flock had been given PTZ in salt mixtures and periodic drenches for 10 years. Drudge was aware that, in drenching his sheep with PTZ liquid, the man would put the appropriate volume of medication for each individual sheep in a glass Coke bottle. The open end was then placed in the mouth of the sheep which swallowed the trickling deposit of drug. Thus, it was a valid assumption that the dead lambs had received

the drug. In other words, it was unlikely that some of the liquid had been lost in administration.

After the initial finding of drug (PTZ)-resistant *H. contortus* in sheep (Drudge et al., 1954; 1957a, b, c; 1958; 1959), Drudge and his colleagues continued almost 50 years of study on this subject. This research included establishing four pasture lots of identical size and terrain. *Haemonchus contortus* was established in sheep on the lots—two lots with PTZ-susceptible and two with PTZ-resistant strains of the parasites. Low-level PTZ was fed to sheep in one of each of the lots with the two worm strains, and there was a nontreated control lot for each category. Further details will not be summarized here, but Drudge’s sheep research on drug-resistant *H. contortus* helped focus on similar problems with horse parasites.

Phenothiazine Initial Activity on Strongyles in Horses

Drudge in 1956 summarized literature on the prophylactic or low-level use of PTZ for control of strongyles in horses. He attributes this usage in horses to that of Shorb and Habermann (1940) who found activity using this method for sheep nematodes. Drudge (1956) states that Dimock (1949) modified and developed the low-level dosage system for horses in central Kentucky. The method used was to feed 2 gm of PTZ daily to adult horses for the first 21 days of each month on a year-round basis. Gibson (1945, 1949) also reported on the benefit of strongyle control by feeding small daily dosages of PTZ. Todd (1952), Todd et al. (1949, 1950b), and Hansen et al. (1949) did research for many years on low-level PTZ feeding of horses in Kentucky. When these researchers went to other universities and Drudge came to UK, he continued these studies (Drudge et al., 1953, 1955). Some of the results of the aforementioned studies with low-level PTZ were that there was minimal toxicity in horses, strongyle eggs per gram of feces (EPGs) remained low, and postmortem examinations revealed large numbers of small and large strongyles indicating inhibition of eggs (more later on this) but not removal of worm specimens.

Drudge, in his 1956 paper, said that the low-level PTZ system was the best method for controlling horse strongyles. He did mention reports of ineffectiveness of the system but believed this was due to faulty administration of the drug. However, already aware of the problems with drug resistance with this regimen in sheep parasites, he noted the possibility of “drug-resistant strains of strongyles” developing, “particularly in view of the apparently favorable conditions in which the worms are more or less in continuous contact with relatively small concentrations of the drug.” He also mentions that “[a]lthough such an unfortunate development is regarded as a possibility, there is no evidence to date that it has occurred.”

It was believed that the beneficial usage of PTZ was mainly that of inhibiting egg production and sterilizing the eggs of strongyles (Gibson, 1945; Drudge et al., 1953). Gibson (1950), in his paper on critical tests of PTZ as an anthelmintic for horses, stated that it had “become evident” that the “inhibition of egg production by worms may follow anthelmintic medication.” This phenomenon had first been recognized by Mhaskar, who, Gibson noted, “showed that the administration of oil of chenopodium inhibited the production of eggs of hookworms in man for as long as 12 days.” Thus, this drug helped lower transmission of these parasites. Todd et al. (1950a) stated that strongyles can account for passage of huge numbers of eggs in horse feces; they estimated that 40 horses could pass more than 500 million strongyle eggs per day. Gibson (1945) showed that egg production by the strongylid worms of horses may be inhibited for as long as 35 days after the end of treatment with 30 1-gram doses of phenothiazine. Also, Gibson (1953) did a classical and probably never repeated study regarding longevity of strongyles when no obvious reinfection occurs. For six aged horses, kept in loose box stalls and periodically treated with 30 gm of PTZ, many treatments had to be given over three years to reduce strongyle EPGs to a low level. This study is often referenced relative to the longevity of strongyle infection.

Phenothiazine Resistance of Small Strongyles in Horses

The first reports of resistance of small strongyles in horses to a compound were with PTZ (Poynter and Hughes, 1958; Gibson, 1960; Drudge and Elam, 1961). The Drudge and Elam (1961) research involved nine studies on three central Kentucky Thoroughbred horse farms during 1960 and 1961. Horses on these farms were treated often with therapeutic dose rates of PTZ but never with the low-level dose rate of this drug. The resistance was determined only from clinical investigations. According to culture data (Drudge and Elam, 1961), PTZ resistance was both for small and large (*Strongylus* spp) strongyles.

Identification of small strongyles was not established in horses treated with PTZ until 1953. This was done by Drudge et al. (1955) who found nine species of small strongyles (*Coronocyclus* [*Cor.*] *coronatus*, *Coronocyclus labiatus*, *Cyathostomum* [*Cya.*] *catinatum*, *Cylicocyclus* [*Cyc.*] *nassatus*, *Cylicocyclus leptostomus*, *Cylicostephanus* [*Cys.*] *calicatus*, *Cylicostephanus longibursatus*, *Cylicostephanus minutus*, and *Petrovinema* [*Pet.*] *poculatus*) surviving low-dose PTZ treatment in two horses. In the same study, an additional three species (*Coronocyclus labratus*, *Cylicodontophorus* [*Cyd.*] *bicoronatus*, and *Gyalocephalus* [*G.*] *capitatus*) were found in the two nontreated control horses. Also, examination of the uteri of small strongyle females revealed eggs present in 1.4% and 7.8% of the specimens from the treated horses, respectively, and in 60.7% and 88.4%

of the specimens of the nontreated horses, respectively. In the treated horses, only two species (*Cya. catinatum* and *Cys. calicatus*) harbored eggs. Drudge states that the latter finding shows that some species of small strongyles still had reproductive capability in spite of PTZ exposure, suggesting tolerance to the drug.

Thiabendazole—General

Thiabendazole, in the early 1960s, was the first of the benzimidazole (BZ) class of compounds marketed. Others in this class followed. These were the first broad-spectrum single-compound drugs active on nematodes in several hosts.

Thiabendazole Resistance of *Haemonchus contortus* in Sheep

In 1961, Drudge designed a study on *H. contortus* and other endoparasites in 40 lambs (10/group) (Drudge et al., 1964). There were three groups treated (TBZ, ruelene, or PTZ) once monthly for six months and one nontreated group. Rapid resistance of *H. contortus* to TBZ (never used before) was noted after the third treatment. Speculation was that perhaps PTZ set up the TBZ resistance because of a similar mode of action of the two compounds (Rew and Fetterer, 1986). Conway (1964) also had an early report of TBZ-resistant *H. contortus* in sheep.

Thiabendazole and Additional Compound (Benzimidazoles, Pyrantel Pamoate, and Piperazines) Resistance of Small Strongyles in Horses

The second parasiticide to which small strongyles were found to be resistant was TBZ. Drudge and Lyons (1965) found that TBZ, when first used on a farm (Farm B) in 1962, was highly effective on small strongyles but, by 1965, resistance of these parasites was evident from lowered reduction of EPG counts. Previously, on this farm, PTZ at therapeutic dose rates became ineffective on small strongyles. There was again speculation about a possible relationship of a common resistance factor of the two compounds because of a similar mode of action, but there was no experimental evidence. Later, Round et al. (1974) also reported small strongyle resistance to TBZ.

Between 1959 and 1983, field studies were done on strongyles in Thoroughbred yearlings and mares on Farm B (mentioned above) in central Kentucky (Drudge and Elam, 1961; Drudge and Lyons, 1965; Drudge et al., 1988, 1990). This research demonstrated that small strongyles (designated as Population B from the farm of origin) were resistant not only to PTZ and TBZ but also to piperazine (PPZ) and pyrantel pamoate (PRT).

Population-B small strongyles were established in 1966 on a University of Kentucky pasture devoid of horse parasites. This was done by the donation of two Farm B

Thoroughbred mares which seeded the pasture with small strongyle eggs. A small breeding band of horses was placed on this pasture, and numerous field studies and critical tests evaluating several compounds were done over about 40 years on the original and succeeding generations of horses born there. The last research on Population-B small strongyles was published by Tolliver et al. (1993) and by Lyons et al. (2007). The first critical tests evaluating activity of TBZ against this population indicated resistance of five (*Cor. coronatus*, *Cyc. nassatus*, *Cya. catinatum*, *Cylicostephanus goldi*, and *Cys. longibursatus*) of the 11 species present (Drudge et al., 1977). In the last critical tests, these five species, in addition to two other species (*Cys. calicatus* and *Cys. minutus*) of the 16 species present, were considered resistant to TBZ. Of these seven TBZ-resistant species, lowest activity was for five by PTZ and fenbendazole (FBZ). Removal of small strongyles was excellent for PRT, oxfendazole (OFZ), and oxibendazole (OBZ) but much less for TBZ, PTZ, and FBZ. It was found that after 22 years of nonexposure to any drug, small strongyles were at a similar level of TBZ resistance as they had been initially. A satellite group of horses infected with Population-B small strongyles was moved to a separate pasture in 1987. Pressure was put on the small strongyles by TBZ treatment until five years before the last critical tests were done. TBZ resistance increased, and one additional species (*Cyc. leptostomas*) was found. Therefore, eight Population-B species were considered BZ-resistant. It is not surprising that these eight species were drug resistant because Ogbourne (1978) reports the 10 most common species as *Cor. coronatus*, *Cya. catinatum*, *Cyathostomum pateratum*, *Cylicocyclus insigne*, *Cyc. leptostomus*, *Cyc. nassatus*, *Cys. calicatus*, *Cys. goldi*, *Cys. longibursatus*, and *Cys. minutus*.

Another long-term study on drug-resistant small strongyles (Population S) at the University of Kentucky was started in 1974 and continues today. Six BZ-resistant species (*Cor. coronatus*, *Cya. catinatum*, *Cyc. nassatus*, *Cys. calicatus*, *Cys. goldi*, and *Cys. longibursatus*) and one PRT-resistant species (*Cys. minutus*) have been documented (Lyons et al., 2001).

Chapman et al. (1991) reported seven of eight of the same species of small strongyles reported by Lyons et al. (2007) as resistant to the BZs; *Cyc. leptostomus* was not included. Wescott et al. (1982) found one additional species (*Cylicocyclus brevicapsulatus*), and Eysker et al. (1988) added two more species (*Cor. labratus* and *Cyc. insigne*) that were resistant to the BZs. Burger and Bauer (1987) identified, for the first time in Europe, seven species which were pro-BZ-resistant, and they were the first in the world to report BZ-resistant *Pet. poculatus*.

Drug resistance of small strongyles has been documented worldwide in numerous instances where parasitocides have been used extensively. After the first reports of resistance of small strongyles to PTZ and TBZ, this situation was reported by the late 1980s for PTZ, all BZs, PRT, and PPZ in the United States and many other countries (Kaplan et al., 2004; Lyons et al., 1999; Pook et al., 2002). It is of interest that the organophosphate dichlorvos (pellet formulation), no longer on the market, was highly effective against BZ-resistant small strongyles (Lyons et al., 1999).

Macrocyclic Lactone (Ivermectin and Moxidectin) Reduced Activity on Small Strongyles and Ascarids in Horses

Small Strongyles

Ivermectin (IVM) and moxidectin (MOX), when first marketed, were highly effective on small strongyles, even those resistant to the other commercially available compounds. There are recent reports that small strongyle EPG counts are returning more quickly than initially after IVM and MOX treatment of equids (Little et al., 2003; Lyons et al., 2008b; Trawford et al., 2005; Molento et al., 2008; von Samson-Himmelstjerna et al., 2007). In six studies on one farm in Kentucky (Lyons et al., 2008b), small strongyle EPG counts began recurring at about four weeks, and they progressively increased at six to eight weeks after IVM treatment of foals and yearlings. The return of the EPG counts was about twice as fast as when IVM was used initially (Boersema et al., 1996; Lyons et al., 1992). Recent data from a low number of critical tests on luminal stages in the large intestine of small strongyles in ivermectin-treated horses on one farm indicated removal of adults was excellent, but on immatures (fourth stage and probably young adults) it was less effective (36-80%) (Lyons et al., 2009).

Ascarids

Ivermectin and moxidectin initially were very effective on *P. equorum*. However, resistance of this parasite to these compounds has been reported numerous times and in many parts of the world (Boersema et al., 2002; Craig et al., 2007; Hearn and Peregrine, 2003; Kaplan et al., 2006; Lindgren et al., 2008; Lyons et al., 2006, 2008b; Molento et al., 2008; Schougaard and Nielsen, 2007; Slocombe et al., 2007; Stoneham and Coles, 2006; von Samson-Himmelstjerna et al., 2007). Anecdotally, there were reports of ascarid resistance to IVM several years ago (Lyons, personal communication). In a recent field study in Kentucky, average reduction (%) in the number of foals passing ascarid eggs in their feces after treatment was as follows: OBZ, 94%; FBZ, 84%; PRT (1x dose rate), 0%; PRT (2x dose rate), 23%; and IVM, 0% (Lyons et al., 2008a). Further research using critical

and/or controlled tests to verify or supplement the field data should be done. Sometimes egg-positive or egg-negative fecal samples after treatment can be misleading. This is because there is potential egg suppression or acceleration after treatment and possibly also slow removal of egg-laying worms. Kaplan et al. (2006) did controlled tests that proved that ascarid-egg-positive horse feces after IVM treatment were a true indicator of lack of drug activity. The low activity of PRT in the Lyons et al. study (2008a) is in contrast to high efficacy found recently by Reinemeyer (2007) in controlled tests with this compound against IVM-/MOX-resistant ascarids in horses. Also, Lindgren et al. (2008) and Schougaard and Nielsen (2007) found effective activity by pyrantel embonate and FBZ against ascarids for which IVM was inactive. Additionally, von Samson-Himmelstjerna et al. (2007) found pyrantel embonate efficacious against IVM-resistant *P. equorum*.

Genetic Features of Ascarids in Horses

It is unknown whether there currently are population differences or some other factors in *P. equorum* removal, or lack thereof, by certain drugs. Extensive genetic and some molecular studies of horse ascarids have been made. More of this type of research should better help to understand the biology of these parasites.

It seems that there is a great need to study the genetic/molecular aspect of horse ascarids in relation to drug resistance/susceptibility in particular. These parasites have an unusually complex genetic composition. For instance, van Beneden in 1883 and later others, according to Cremer and Cremer (2006), reported that *P. equorum* (then called *Ascaris megalcephala*) specimens have either two (univalent) or four (bivalent) chromosomes. Bullini et al. (1978) indicated that there are actually two *Parascaris* species: *Parascaris equorum* ($2n = 4$) and *Parascaris univalens* ($2n = 2$). They found that these species showed substantial genetic divergence based on enzyme electrophoretic studies (allozymes) (method clarified to current authors by Steve Nadler, personal communication). Biological supply companies used univalent and bivalent horse ascarids (they called them *A. megalcephala*) for many years to make microscopic slides showing mitosis and meiosis for biology students (Anon., 1968). In the late 1950s and early 1960s, Drudge and Lyons provided uteri from horse ascarid specimens to biological supply companies; some of these specimens were examined and found to be bivalent. Li (1934) said that Boring in 1909 reported both univalent and bivalent ascarids in the same horse. Chitwood and Chitwood (1950) quote Li (1934, 1937) as finding these parasites with both six and nine chromosomes in Chinese Mongolian horses; the six-chromosome species (some

might say subspecies) of ascarids were called *Parascaris equorum trivalens*. It is evident that there are various reasons for studying horse ascarids more thoroughly.

Another interesting feature of horse ascarids is that they played a major historic role in genetics. According to Pimpinelli and Goday (1989) and Hamoir (1992), van Beneden in 1883 in his research on *P. equorum* (*A. megalcephala*) was the first to report meiosis at the chromosome level. This was on his discovery that gametes of these parasites contain only one-half the chromosome complement of somatic cells. The finding of van Beneden was probably influenced from earlier research by Hertwig in 1876 on sea urchin eggs (Hamoir, 1992).

Summary

This bulletin describes certain aspects of small strongyles and ascarids in horses with notes on *H. contortus* in sheep. The main emphasis is on drug resistance and especially research at the University of Kentucky. It highlights the pioneer, Hal Drudge, DVM, ScD, and his almost 50 years of research on this subject, alone or with colleagues, at UK. He was the first to do research and publish on drug resistance of a nematode species. This was on phenothiazine resistance of the “barber pole” stomach worm (*H. contortus*) in sheep. Later, Drudge and others in England found small strongyles resistant to this drug in horses. Thiabendazole was highly active initially against small strongyles and *H. contortus*, but resistance was found later in Kentucky and other geographical areas. A fairly short time after their extensive usage, benzimidazoles (e.g., thiabendazole), pyrimidines (e.g., pyrantel pamoate), and piperazines became ineffective against small strongyles. This phenomenon has been studied continuously for several decades at the University of Kentucky and continues now (2009). Numerous other researchers in many parts of the world have documented the resistance problem with these compounds for control of these parasites. Recently, research in various locations, including Kentucky, has recorded that small strongyle EPGs in horses treated with macrocyclic lactones (IVM or MOX) are returning sooner than when the drugs were first marketed. At the University of Kentucky (Lyons et al, 2009), critical tests in horses treated with IVM revealed that removal of small strongyles in the lumen of the large intestine was 100% for adults and 36 to 80% for immatures. Results of this study indicate the following probable reason EPGs of these parasites now are returning sooner than initially in horses treated with IVM. Apparently, lessened activity on immatures in the lumen of the large intestine has led to a shorter “completion” of the life cycle. Ascarids are now resistant to activity of IVM and MOX according to research in various locations in the world, including Kentucky.

Overview

As mentioned above, most chemical classes of parasitocides have become inactive, especially against nematodes, after a period of use in domestic animals. This has resulted in a major dilemma because no new broad-spectrum classes of chemicals have been marketed for nematode control since the avermectins in the early 1980s. Adding to the situation is the apparent lack of new compounds becoming available commercially in the near future. Besides lowered efficacy of drugs, the number available for horses has declined substantially in the past 20 or so years. It is obvious that there has been too much reliance on, and in many cases, unnecessary overuse of drugs. For instance, for strongyle control, profiles of EPG counts for individual horses could be established and then only the horses with high EPG values could be selectively treated. In other words, don't routinely treat all horses on a farm for strongyles. Currently, there is more leeway with strongyle control because the many years of usage of the benzimidazoles/macrocyclic lactones/pyrimidines have dramatically reduced the prevalence of the most pathogenic nematodes

(*Strongylus* spp) in horses on farms with routine deworming programs. This situation could change if drug resistance of these species occurs or if treatment programs change. Under current circumstances, parasite control is in general a "throw-back" to over 50 years ago before the advent of effective drugs. This essentially means that, with exceptions where drugs are still effective, horse parasites cannot be as controlled now as in the past several decades. It is highly important that practical measures other than, or in addition to, using drugs need to be developed and implemented to aid in parasite control.

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