

ALTERNATIVE METHODS FOR THE CONTROL OF GASTROINTESTINAL PARASITES IN HORSES WITH A SPECIAL FOCUS ON NEMATODE PREDATORY FUNGI: A REVIEW*

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Abstract

The paper describes a review of alternative methods of horse parasite control. Horses serve as hosts for a wide array of parasites, among which strongylid nematodes (Strongylidae) constitute considerable pathogens. Due to the short and monoxenous life cycle of these parasites and also the resistance to benzimidazole and other drugs, the control strategies are often ineffective. Thus, the treatment of strongylids constitutes a priority aiming at the improvement of animal welfare, with special attention paid to the stable and pasture organization. The treatment carried out in the stable should consider the frequent exchange of horse bedding at regular time intervals, periodic disinfection of stalls and maximum limitation of contact of feed and water with the stable substratum. An application of feed supplements, i.e. plants or plant extracts, which act as natural dewormers, also constitutes an important factor in the control of parasitic infections. Among the treatments applied to the pasture, the regular removal of waste, quarter pasturing, as well as an alternate pasturing with other animal species, are of special importance. The use of deworming properties of fungi, which constitute a natural component of green growth in pasture, seems to be the next method of nematode control, already confirmed by several studies.

Key words: horses, parasites, control, pasture management, strongylid nematodes, alternative methods

Parasitological hazards in horses

Horse parasitoses pose economic and health risks that are of concern to both breeders and horse owners. Insufficient knowledge of antihelminthics often leads to improperly conducted, and thus ineffective, horse deworming (Nielsen et al., 2006).

Equidae are hosts to a variety of helminths, of which Strongylidae constitute a particularly important group of parasitic nematodes. Strongylidae commonly oc-

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cur in the intestines of horses of all breeds and can be found in both sexes and all age classes, though young horses are more susceptible (Van Doorn et al., 2012). Roundworms (*Parascaris equorum*) and pinworms (*Oxyuris equi*) are most often found in foals and adult horses, respectively. Young mares and stallions can also carry cestodes. Asses, zebras and other wild equines may exhibit a greater resistance to parasitic infection and less pronounced clinical symptoms relative to domestic horses (Malan et al., 1997). Furthermore, wild horses are less likely to suffer from parasite-induced diseases when infected with helminths. In horses, parasitic infections may manifest as clinical symptoms, changes in the morphological and biochemical characteristics of blood or pathological changes in internal organs (Love et al., 1999). As early as 150 years ago, intestinal parasites were associated with diarrhoea (Knox, 1836). Some publications have described an association between the occurrence of small strongyles with diarrhoea and/or body mass losses (Love, 1992) or colic (Uhlinger, 1990). Instances of death have also been observed (Assis and Araújo, 2003). However, the majority of parasite infections go unnoticed, and the occurrence of disease is the exception rather than the rule.

The most frequently used method for controlling internal parasites is the application of antihelminthic drugs (Braga et al., 2010). Although twice yearly deworming of free-ranging horses can eliminate warble infestations and tapeworm infections, the control of strongyles is much more complicated (Gawor and Kita, 2006). The routine deworming of horses in the spring is only effective in the short term because reinfections can rapidly occur in yards or pastures. Kornaš et al. (2008) showed that nematodes occur seasonally. According to these authors, after April deworming in European climatic conditions, the parasite count in faeces is the lowest in June and increases in October. Additional problems include the emergence of drug resistance and the fact that antihelminthics only affect adult small strongyle worms (Kaplan, 2002). The first reports on resistance to benzimidazole drugs appeared in the 1960s (Drudge and Elam, 1961), and pyrantel resistance was reported in the 1990s (Chapman et al., 1996). Recently, a shortening of the small strongyle egg reappearance period following the application of ivermectin was reported (Lyons et al., 2008), which may indicate the emergence of macrocyclic lactone resistance (Lyons et al., 2009). The resistance has a genetic background and is favoured by the short developmental cycle of these nematodes. Drug resistance does not occur among large strongyles (Gawor, 2006).

The need for new, non-chemical methods of nematode control in horses and other farm animals has recently been discussed as a response to the problems mentioned above. It has been demonstrated that, in addition to the problem of drug resistance, chemical agents also influence meat quality (Peters et al., 2009). In addition, the negative impact of antihelminthics on the environment in addition to human and animal health is an issue of significant importance (De and Sanyal, 2009).

Management solutions

Alternative methods of controlling horse parasites are environment targeted, with pastures and stables being settings of particular importance. Proper pasture management is a valuable approach for decreasing the transmission of parasites, particu-

larly because pastures are the main sources of gastrointestinal nematode infections (Barger, 1999). Barger (1999) provided a number of valuable insights in this regard. Management is defined as the use of alternative horse pasturage strategies at various ages that prevent acute parasitic infections in young animals (Von Samson-Himmelstjerna et al., 2009). Pasturage of horses with other farm animals (e.g., sheep, goats or cattle) is also acceptable because it leads to the interruption of the developmental cycle of parasites of each of the animal species concerned (Dimander et al., 2003). Selecting an adequate assortment of animals for a given pasture is also important (Fritzen et al., 2010). Horses should not graze in the morning or at dusk because the density of the larvae in pastures is greatest during these times (Klei and Baudena, 1999). Horses should also not be allowed to graze in pastures that were used intensively in the previous year because invasive larval stages may persist over the winter (Ribbeck et al., 1997). Grass in pastures should be regularly mown because small strongyle larvae are capable of vertical migrations along grass blades, positioning themselves on the tops of the plants and thus accelerating infection (Bauer and Hertzberg, 2002). Michel (1985) further defined pasture management as consisting of preventive, evasive and weakening practices. Preventive practices include the introduction of dewormed animals onto clean pastures. If such an approach is not possible, anti-parasitic measures should be taken at an early stage of the pasturage season until a time at which the initial population of invasive larvae on the pasture has been reduced to a safe level. This strategy is used by Australian sheep breeders. Anthelmintics are used during weaning, and lambs are transferred to pastures that are either clean or have been grazed by parasite-resistant animals. Anthelmintics are then used an additional two or three times at eight-week intervals (Barger, 1995). A particularly extreme preventive strategy is the combined grazing of a pasture by two species (e.g., sheep and cattle) with different helminth composition. Parasites characterized by high level of specificity usually fail to infect the alien host. Such alternate grazing strategies should be employed at intervals of 2 to 6 months with the optional use of anthelmintics (Donald et al., 1987). Eysker et al. (1983; 1986) investigated alternate grazing methods using Shetland horses and sheep; in their study, the horses were also dewormed. The results of their study indicated a decrease in the level of Cyathostominae and Strongylinae nematode infections.

Evasive practices are based on the use of anthelmintics in combination with the transfer of animals from contaminated pastures to uncontaminated ones immediately before larvae hatch and transform into invasive forms, which reach a considerable density on primary pastures (Michel, 1976). Weakening practices are based on the pasturage of more than one animal species at the same time to break the developmental continuity of each parasite species, making it difficult for the parasites to find an ultimate host. In addition, the proper maintenance of pastures (e.g., by liming, weight-back mowing, drainage of wet areas and the removal of faeces, which are the main source of helminth infections) significantly reduces the occurrence of parasite infections.

In African countries, horses, asses and mules are used both in agricultural work and for transporting goods and people (Krecek et al., 1994). The stress associated with such work, combined with restricted feeding schedules, results in poor animal

condition and an increased susceptibility to parasitic diseases. The reduced availability and/or high costs of deworming agent application in many African countries means that the typical strategy for preventing parasitic infections is the removal of faeces, which act as egg reservoirs for some parasites (e.g., *Cyathostominae* or *P. equorum*). The removal of faeces breaks the parasites' developmental cycle and reduces the density of invasive larvae in pastures. The faeces obtained are then used as fuel, building material or compost (Krecek and Guthrie, 1999). The presumptive costs of removing faeces from pastures are compensated by the lower treatment costs for colics of a parasitic background, the delayed emergence of drug resistant parasites and an increase in usable animal pasturage area corresponding to the areas cleared of faeces (Hinney et al., 2011). However, such practices require substantial effort, which is likely why breeders still opt for quick and relatively simple pharmacological approaches.

Some horses spend the majority of their time in stables. Thus, maintaining proper hygienic conditions in stables is essential for the prevention of parasitic infections. Horse boxes should be regularly disinfected, particularly before the introduction of new animals. Disinfection of stables should be performed at least twice a year, following the beginning and end of the pasturage season. Disinfectants should be used after all surfaces and equipment have been carefully cleaned (Hasslinger, 1986).

To prevent infections with parasites that may be present in bedding, feed must be provided in mangers and hanging nets and should not be placed on the floor. Cribs and drinking bowls should be clean because they may contain intermediate hosts of some helminths. Regular cleaning of boxes and biothermal manure decontamination are also important (Hasslinger, 1986). Removed manure should be stored at a height of approximately 1.5 m above the ground and should be covered with bedding and a soil layer of at least 20 cm thickness, sprinkled with biocide and left for 42 days. This procedure promotes the disposal of parasite eggs and larvae, thus breaking the helminth developmental cycle (Gundlach and Sadzikowski, 2004).

Nutrition is also relevant to parasite control. Full-value nutrition, through its influence on the specific and non-specific immunity of animals, makes parasitic infection difficult. Increasing the overall immunity of horses via supplementation of feed with vitamins A and D and B-complexes also favours increased resistance to endoparasites. Mineral supplementation has similar effects (Masters et al., 1999). The requirements of horses should be determined via non-invasive examination of the hair coat's elemental composition.

Plant-fodder supplements

Many plant species that naturally grow in pastures exhibit antihelminthic effects; such plants can be fed to horses with feed in mangers. Plants that exhibit deworming properties include: common wormwood garlic (*Allium sativum*) (Benksy and Gamble, 1993), *Cucurbita moschata* (Marie-Magdeleine et al., 2009), *Artemisia absinthium*, eastern black walnut (*Juglans nigra*), *Cucurbita pepo*, mugwort (*Artemisia vulgaris*), fennel (*Foeniculum vulgare*), herb hyssop (*Hyssopus officinalis*) and common thyme (*Thymus vulgaris*) (Burke et al., 2009). These species display various levels of activity towards nematodes and other human and animal parasites.

The antihelminthic activity of *Cucurbita moschata* seeds against *Haemonchus contortus* nematodes, which occur in small ruminants, has been assessed. The methods of Marie-Magdeleine et al. (2009) using water, methanol and dichloromethanol extracts of *C. moschata* appear to be promising. In comparison to a control group, each *C. moschata* seed extract exhibited a highly significant ability to inhibit the development of *H. contortus* larvae.

With respect to the strongylid nematodes discussed above, the efficacy of the bitter plant *Artemisia brevifoli* was also verified (Athanasiadou et al., 2007). It has been shown that consumption of whole plants caused a 62% decrease in the extensiveness of *H. contortus* infections in sheep (Iqbal et al., 2004). In addition, the consumption of leaves from the African tree *Zanthoxylum zanthoxyloides* was found to reduce the prevalence of *H. contortus* in sheep (Hounzangbe-Adote et al., 2005).

Predatory fungi

Interest in biotic factors that are capable of controlling invasive nematode larvae in farm animals first arose in 1930. The biological control of intestinal nematode infections is a seemingly promising approach that is less demanding than antihelminthic treatments. Biological control strategies utilize fungi that naturally occur in the environment and use nematodes as a source of nutrients (Araújo et al., 2008). However, none of the fungi species that have been tested were able to entirely eliminate parasites; instead, they could only decrease the parasitic infections to safe levels at which animals could develop natural resistance to small numbers of nematodes (Larsen, 2006). These fungi were divided into three groups according to their morphology and effect on parasites: (i) predatory fungi, (ii) endoparasitic fungi and (iii) egg-parasitic fungi (Barron, 1997). (i) Predatory fungi have various catching structures or traps, tacky nets, adhesive nodules or tightening rings present on the surface of the mycelium, where the prey is trapped. An example of such fungi that have been discussed in numerous studies is *Duddingtonia flagrans*, which was found to cause a considerable reduction in the number of nematode parasitic larvae in experiments conducted on cattle (Grønvold et al., 1993), sheep (Peloille, 1991) and horses (Baudena et al., 2000). (ii) Endoparasitic fungi can live inside a prey's body and infect it using spores. The adhesive conidia of such fungi produce droplets of viscous substances and attach to the nematode cuticle. They then penetrate the prey's cuticle and assimilate its contents, thus destroying it. Other endoparasitic fungi produce small, half-moon-like conidia that are consumed by nematodes. The conidia remain in the parasite's alimentary tract; after they germinate and develop, they perforate the parasite's cuticle to produce new spores outside. The efficiency of this mechanism has been verified in laboratory conditions by numerous groups, including Charles et al. (1996) who observed a significant reduction in the number of nematode larvae under the influence of fungi *Harposporium anguillulae*. (iii) Egg-parasitic fungi are capable of attacking and destroying helminth eggs. They may be useful in controlling the infection levels of parasites with relatively long developmental cycles and/or a long dispersion time beyond host organisms in the form of eggs (e.g., the typical geohelminths *Ascaris* spp. and *Trichuris* spp.). The effectiveness of this group of fungi, among others, was verified using species of the genus *Verticillium* (e.g.,

V. chlamydosporium). These fungi destroyed *Ascaris suum* eggs obtained from naturally infected pigs; specifically, they damaged the egg shells enzymatically, penetrated the inside of the eggs and absorbed their contents. One example of such an ovicidal fungus is *Pochonia chlamydosporia*, which develops undifferentiated hyphae that colonize egg envelopes and eventually penetrate the egg, resulting in its destruction (Araujo et al., 2009). Braga et al. (2010) tested the effectiveness of the fungus in relation to *Oxyuris equi*, finding that it resulted in a decrease in the invasion of the species.

In developing biological approaches to combat parasites, the aim is to identify fungi whose spores pass through the animal alimentary tract in an intact form, germinate in the faeces, and attack and eliminate the larvae developing in them (Von Samson-Himmelstjerna et al., 2009). Such fungi should be inexpensive to produce, easy to use and safe for animals (Chandrawathani et al., 2002). Unfortunately, some species only exhibit high nematicide potential in laboratory tests and are not useful in natural animal habitats (Larsen, 2006). Grønvold et al. (1993) showed that numerous attempts to verify the effectiveness of *Arthobotrys oligospora* were not successful because its nematicide structures are damaged in the alimentary tracts of animals.

The fungus that best fulfils the requirements described above appears to be *Duddingtonia flagrans*. This species belongs to a heterogeneous group of microfungi that eliminate parasitic nematodes, using them as either a protein source or as a supplement to enable their saprophytic lifestyle (Barron, 1997). This cosmopolitan species is found in a variety of habitats, but is most often encountered in sites that are rich in organic matter, such as compost or animal faeces. The latter seems to be the preferred and most quickly colonized habitat (Saumell et al., 1999). The development of *D. flagrans* is relatively slow and, as with other fungi, temperature-dependent (Fernández et al., 1999). Its chlamydospores, having passed intact through the alimentary tract, are excreted with faeces, where they begin process of germination. Subsequently, they form viscous three-dimensional nets that enable them to trap and eliminate invasive parasite larvae, including strongyles (Larsen, 2006).

The effectiveness of *D. flagrans* has been verified on each continent. However, the existing research has primarily been conducted using sheep, for which the problems resulting from parasite infection are (relative to other animals) more clearly tied to production economics. For example, in Australia, using the increased susceptibility to helminth infection of new individuals and pregnant females, one study included lambs aged six to nine months of age (Urquhart et al., 1998). It was shown that the administration of *D. flagrans* spores to animals in the experimental group at three different doses resulted in a reduction of the nematode infection intensity to almost zero, while in the control group, it varied between 60 and 100% (Waller et al., 2001). A similar experiment was performed in the USA by Pena et al. (2002), who noted a 94% decrease in infection intensity; furthermore, the studies of Terrill et al. (2004) described a decrease of 80% in goats. Equally promising results were obtained by Sanyal et al. (2008) and Silva et al. (2009) in investigations of *D. flagrans* application in sheep.

A number of studies have investigated the effectiveness of *D. flagrans* in the prevention of nematode infection in horses. Fernández et al. (1999) noted a reduction in

invasive L3 larvae in faeces samples by 67–99.7%. These results were influenced by atmospheric conditions and the size of the administered chlamydospore dose. Braga et al. (2009) observed a 46.2% decrease in the average number of eggs (EPG) in the experimental group, while the reduction in the amount of larvae in the faeces of the analysed horses was season dependent, ranging from 51% to 68.5%. Moreover, the control group exhibited a gradual decrease in body weight, in contrast to an increase in body weight observed in the experimental group. A distinct decrease in the number of L3 larvae in pastures influenced by *D. flagrans* in the case of horses and other animal species was also observed by both Wolstrup et al. (1994) and Larsen et al. (1996). A study by Baudena et al. (2000), who in southern USA climate conditions observed a 55% reduction of the number of invasive larvae in coprocultures in the autumn months and a 99% reduction in spring, also confirms the results discussed above.

The nematicide activity of *D. flagrans* is not limited to egg-infested faeces. The range of fungal spores in plants found in the proximity of faeces was also analysed. The reduction of the number of larvae in such plant material ranged from 65% to 95%. A similar reduction (from 78.5 to 82.5%) in the number of larvae in pastures was observed by Braga et al. (2009). In both cases, the reductions resulted from the application of a daily dose of chlamydospores at the level of 10⁶/kg of a subject animal's body mass. The effectiveness of similar preparations had already been described by Larsen (2006). Unfortunately, the practical application of *D. flagrans* is impeded by particular challenges; namely, a lack of ready to use spores and product registration challenges in the European market (Gawor, 2006).

The results of the studies described above clearly prove the high nematicidal activity of *D. flagrans* and support its use in controlling gastrointestinal nematodes (including strongyles) in horses. The discussed results also indicate a need to reconsider the effectiveness and consequences of routine applications of chemical pharmacological agents. Furthermore, the results suggest that, particularly considering the already evident issue of drug resistance (e.g., among Cyathostominae), more attention should be focused on alternative procedures. In the future, such alternative procedures may be the only effective methods of nematode control.

Despite limitations described above, the benefits of alternative, non-pharmacological methods for controlling small strongyle infection levels in horses appear to be substantial. However, the number of studies performed directly on horses is not yet sufficient; furthermore, the labour-intensive nature of some of these approaches, combined with the ambiguous and short-term nature of their financial benefits, mean that such methods are not frequently used by breeders. We believe that this situation must change. The infection of animals with drug-resistant parasites continues to be a pressing problem that must be addressed, not only for the sake of improving animal health, but also for long-term economic reasons. The dynamic development of horse-breeding methods for horse-riding sports, tourism and even hippotherapy, as well as the increasingly popular pro-ecological model of animal husbandry, indicates that breeders will change their approaches for the prevention and treatment of parasitoses.

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Alternatywne metody kontroli pasożytów przewodu pokarmowego u koni ze szczególnym uwzględnieniem drapieżnych grzybów nicieniobójczych: artykuł przeglądowy

STRESZCZENIE

Praca opisuje przegląd alternatywnych (niefarmakologicznych) metod kontroli pasożytów u koni. Konie są żywicielami wielu gatunków pasożytów, z których nicienie zaliczane do tzw. słupkowców (*Strongylidae*) stanowią jedną z najważniejszych grup. Nicienie te są potencjalnym zagrożeniem dla zdrowia koni, a w ekstremalnych przypadkach także dla ich życia. Ponieważ cechują się prostym cyklem rozwojowym, a niektóre z ich także postępującą lekoopornością, ich zwalczanie często okazuje się nieefektywne. W konsekwencji kontrola słupkowców powinna być prowadzona na wielu płaszczyznach, które w szczególności powinny skupiać się na poprawie szeroko rozumianych warunków zoohigienicznych, w których przebywają zwierzęta, ze szczególnym uwzględnieniem organizacji stajni oraz pastwiska. Spośród zabiegów prowadzonych w stajni szczególną uwagę zwraca się na regularne i częste usuwanie ściółki, okresową dezynfekcję boksów czy maksymalne ograniczanie kontaktu pokarmu i wody zwierząt z podłożem stajni. Ważnym elementem kontroli inwazji pasożytów okazało się także stosowanie suplementów paszowych w postaci roślin lub ich wyciągów o potwierdzonych właściwościach odrobaczających. Do ważniejszych zabiegów prowadzonych na pastwisku należy cykliczne usuwanie odchodów, wypas kwaterowy oraz wypas naprzemienny koni z innymi gatunkami zwierząt. Dodatkowo, potwierdzoną już wieloma badaniami, możliwością kontroli nematodów koni okazało się także wykorzystywanie nicieniobójczego działania naturalnie występujących w runi pastwiskowej grzybów.