

Anthelmintic resistance in horse cyathostomins in Europe: current status and future perspectives

Donato Traversa

Department of Comparative Biomedical Sciences, University of Teramo, Piazza Aldo Moro 45, 64100 Teramo, Italy.

Correspondence: Tel +39 (0) 861 266870; Fax +39 (0) 861 266873; Email dtraversa@unite.it

Abstract. Adult and larval stages of cyathostomins (Nematoda, Cyathostominae), known also as “small strongyles”, cause different degrees of intestinal distresses and diseases in horses. In the past decades, drug resistance in cyathostomins has become widespread, especially for benzimidazoles and tetrahydropyrimidines. The recent reports of reduced efficacy in macrocyclic lactones are also of great concern. Given the recent advances into diffusion and distribution of drug resistant populations in different European areas, the present article has the aim to review the current status of the knowledge of anthelmintic resistance in horse cyathostomins in Europe and to discuss perspectives for implementing current control measures.

Keywords: Cyathostomins; Horse; Europe; Anthelmintic resistance.

Received 18/01/2010. Accepted 03/02/2010.

Background

The past decades have been dominated by a renewed interest in the biology, epidemiology and pathological significance of intestinal parasitic nematodes known as “small strongyles” or “cyathostomins”. Cyathostomins play a relevant pathogenic role in horses, causing clinical signs like lethargy, sudden weight loss, debilitation, and diarrhea (Uhlinger, 1991; Murphy and Love, 1997; Mair et al., 2000). Additionally, larval stages can be even more dangerous since at the onset of their invasion of the host, the third larval stages (L3) encyst in the gut wall where may cause serious damage to the mucosa. Thousands of encysted larvae may, in turn, cover the wall, severely damaging it and reducing nutritional metabolism. When a high number of the developed fourth stage larvae (L4) emerge

from the wall into the lumen to continue their life cycle to the adult stage, they cause a condition known as “larval cyathostominosis”. This syndrome, due to severely damages to the large intestine, is mainly characterized by diarrhea and potentially serious colic, with a mortality rate as high as 50% (Giles et al., 1985; Eysker et al., 1989; 1990; Love and McKeand, 1997).

Except the pathogenic role, the attention given to these nematodes has greatly grown due to the difficulties that have emerged and are presently worldwide emerging in their management and control (Lyons et al., 1999; Kaplan, 2004). The problem of anthelmintic resistance in horse cyathostomins is widely reported in the literature from Europe and overseas as well, and the existence of drug resistant populations represents a major

problem for equine health and welfare. More specifically, resistance to benzimidazoles (BZ) is spread all over the world, and resistance to tetrahydropyrimidines (THP) is increasing in prevalence in different countries. While macrocyclic lactones (ML) remain the most effective compounds, by 2007 the first cases of reduced efficacy have been reported in the Americas and Europe (for a review see Traversa et al., 2009). Therefore, there is now a great interest by horse parasitologists in new and integrated control methods aiming both to control cyathostomins and to preserve anthelmintic effectiveness as long as possible. In the light of the new difficulties in using anthelmintic drugs against small strongyles, the basic step to improve control and prevention measures is represented by increasing awareness and knowledge on the epidemiology of these parasites and on the presence and diffusion of susceptible and resistant populations across different territories. Therefore, the aim of the present review is to report the current status of the knowledge of anthelmintic resistance in horse cyathostomins in Europe and to discuss perspectives for further studies towards effective control measures.

Drug resistant cyathostomins in Europe

After the first reports from the 1970's, several other publications in different European countries have appeared in the scientific literature. Indeed, in Europe cyathostomins resistant to one ("single resistance") or more ("multiple resistance") anthelmintic classes have been reported in a range of countries (reviewed by Traversa et al., 2009). For instance, the prevalence of resistance to BZ in cyathostomins in Switzerland was shown to be greater than 50% of the examined herds (Meier and Hertzberg, 2005) and prevalence rates up to 100% were reported in Sweden (Nilsson et al., 1989), Denmark (Bjorn et al., 1991), England (Fisher et al., 1992), Slovak Republic (Varady et al., 2000), Germany (Wirtherle et al., 2004) and Romania (Cerneia et al., 2008). Similarly, resistance to THP, though less prevalent, has been described both in Europe (Ihler, 1995; Craven et al., 1998; Osterman Lind et al., 2007; Traversa et al., 2007) and USA (Champan et al., 1996; Kaplan

et al., 2004). Despite the importance of resistance in horse cyathostomins, there are countries in which studies on drug resistance has only been evaluated relatively recently. This is the case of Italy, where the first study was carried out in 1992, when cyathostomin populations resistant to probenzimidazoles (PBZ) and mebendazole were found in three farms of northern Italy (Genchi et al., 1992). The subsequent survey was carried out 14 years later, to determine the distribution and prevalence of drug resistant cyathostomins in two Italian areas. This last survey indicated that fenbendazole (FBZ) and/or pyrantel (PYR) resistant cyathostomins are present in Italy, even though with a prevalence appearing to be less than that described in other European regions (Traversa et al., 2007).

Nonetheless, given that the vast majority of these studies relied on a small number of horse yards in limited areas, there was a certain merit in geographically and numerically broader investigations of drug resistance on horse populations from Europe. The lack of extensive data on the presence and distribution of drug resistance spurred a large scale survey carried out in 2008 to evaluate the efficacy of the major drugs used in current equine practice and the prevalence of resistant parasite populations in Europe. This survey investigated, according to the current definitions of resistance by the World Association for the Advancement of Veterinary Parasitology (WAAVP), the efficacy of FBZ, PYR, ivermectin (IVM) and moxidectin (MOX) in a total of 102 yards/1704 horses from three countries: 60 yards/988 horses from Italy, 22/396 from the UK and 20/320 from Germany. The testing of FBZ on a total of 80 yards resulted in resistance present on more than 80% of the UK and German yards and on significantly fewer in Italy (~ 38%). PYR was tested on a total of 102 yards and resistance present was found in about 25% yards with no significant differences between countries. Resistance to IVM was detected in one Italian and two UK yards, while resistance to MOX was not declared in any yard in any country. This study, the largest ever carried out on the diffusion of drug resistant cyathostomins, showed that single and/or multiple drug resistance in small strongyles of horses is

established in the three examined countries, with higher prevalence for FBZ followed by PYR. In some cases, multiple resistance to FBZ and PYR was observed. ML proved to be the most effective drugs, with few evidences of efficacy reduction for IVM and highest activity of MOX, despite a single case of reduced efficacy in Germany (Traversa et al., 2009).

Control measures: current status and future perspectives

The picture showed by the last studies carried out in Europe shows that a single-dose use of BZ might be ineffective against cyathostomins in dozens of horse farms. PYR is also losing efficacy in different European countries and the efficacy of ML remains high. Nonetheless, there are countries in Europe (i.e. Italy, UK, Germany) and in the USA (i.e. Kentucky) as well, where the efficacy of IVM has been already observed to be <90%, which can be considered an indicator of upcoming resistance. Although MOX was recently reported to no longer provide control of cyathostomin infection in an area of Brazil (Molento et al., 2008), the molecule is 100% effective in treating the infection caused by small strongyles in Europe, given that the only exception was a single horse from Germany in the survey carried out in 2008 (Traversa et al., 2009).

The clear presence of cyathostomin populations resistant to different anthelmintic compounds across European countries calls for the development and implementation among veterinarians, owners, farmers and managers, of further plans to reduce the expansion of resistant populations and to use the parasiticides that remain effective in a manner that preserves their efficacy as long as possible. It is also important to consider integrated control measures as part of the normal worm control programs. For instance adequate pasture hygiene, low stocking rates, age class division and mixed grazing with other animals are key measures to be integrated with the administration of drugs.

The proper use of anthelmintic drugs is fundamental in controlling small strongyles. In fact, erroneous practices such as underdosing,

over-use, lack of accurate weighing of the animals to be treated and off label use of treatments in animals are the basis of the selection of resistant parasitic populations. For instance, the differences in prevalence of resistance to THP between the US and most of the rest of the world, was explained in the past by the common practice of daily feeding of low-dose PYR tartrate in North America. This might, in theory, have a strong impact in the selection for resistance also to other PYR salts (Kaplan et al., 2004). However, the levels of reduced efficacy of PYR found in Europe, where the daily feeding is not practiced, demonstrate that such a program is not necessary for selection for resistance (Traversa et al., 2009). Another relevant example is represented by the widespread loss of efficacy of BZ and PYR in UK, where over-treatment of thoroughbreds in the past, likely caused the spread of worm populations resistant to these classes of compounds (Comer et al., 2006).

Horse properties need to be regularly monitored not only for resistance to BZ and PYR, but also to ML. Over-use of IVM and MOX should be avoided in those farms where the other anthelmintics are still effective. Specifically, the drug resistance status must be yearly investigated by the Fecal Egg Count Reduction Test (FECRT) on each farm and when effective, BZ and THP should be administered to reduce the pressure on ML (Kaplan et al., 2004; Molento et al., 2008). Additionally, the constant monitoring of anthelmintic effectiveness needs to take into consideration the egg reappearance period of IVM, which is commonly used for quarantine treatment for newly introduced horses (von Samson-Himmelstjerna et al., 2007; Molento et al., 2008). If animals treated with IVM shed cyathostomin eggs after 4 weeks rather than 6 to 8 weeks, the status of the animal is worth further evaluation with sensitive quantitative copromicroscopic evaluations like the McMaster technique or the more sensitive Stoll's method (Kaplan et al., 2004; Molento et al., 2008). This is even more important, considering that in the past few years the cyathostomins eggs reappearance period after IVM treatments showed to be shortening in Germany (von Samson-Himmelstjerna et al., 2007).

Other “new” indications to further control horse small strongyles are based on the use of “selective treatments” rather than “interval dose program” (i.e. treat-all-animals). This reflects a pivotal point in diluting resistant genes in parasitic populations and the maintenance of the refugia. The preservation of the refugia is based on the identification and treatment of animals actually needing an anthelmintic treatment, thus avoiding the treatment of animals that would be instead treated more frequently than necessary (i.e. over-use of parasiticides). This approach is based on the knowledge of the FEC values in the animals, as the indicative minimum cut-off of 200 cyathostomin eggs per gram of feces (EPG) has been recommended as a guide to the need for treatment of individual horses (Coles et al., 2003; Kaplan et al., 2004; Molento et al., 2008). In this manner, the aims of controlling cyathostomin-induced health problems while simultaneously reducing pasture contamination and preserving a pool of susceptible parasites should be achieved.

Worthy of note is that, usually, in a farm, only a small proportion of the infected horses shed a high level of small strongyles eggs. These are the animals that should be treated according to the selective approach. Hence, leaving a proportion of horses untreated would, in turn, maximize the refugia, with a small impact on the health control program, as horses with low egg count can be considered not important sources of environmental contamination. Furthermore, the eggs eventually shed by animals possibly infected with resistant populations would be diluted in the pastures by the eggs shed from untreated animals. In this way the selection pressure would be progressively reduced (Kaplan et al., 2004; Molento et al., 2008).

Concluding remarks

The overall outlook presently known for Europe undoubtedly enhances the concern regarding the spread of anthelmintic resistance in horse cyathostomins. The recently generated data (Traversa et al., 2009), on the other hand, provides some basic knowledge to prompt and improve awareness of drug resistance and to stimulate the rational use of parasiticides in

programs that minimize selection for loss of efficacy.

Several new drug classes, such as cyclooctadepsipeptides, paraherquamide, amino-acetonitrile derivatives, are powerful anthelmintics and some of them are already on the market (Zinser et al., 2002; Harder et al., 2003; Kaminsky et al., 2008) but their potential applicability to horse cyathostomins is unknown. Therefore, if the extent of resistance to ML increases and spreads, horse owners and equine practitioners will be faced with an important problem with no immediate solution. Since resistance to ML may worsen or may be spread by horse movement, there is an urgent need to appropriately use the remaining effective drugs, especially MOX, in order to preserve their efficacy. Given the strong impact that resistant cyathostomins can and will likely have on the health horses, future parasite control plans should be based on integrated measures represented by correct use of anthelmintics and other approaches, such as adequate pasture hygiene, low stocking rates, biological control by nematophagous fungi, and mixed grazing with other animals (Waller, 1987; Lloyd et al., 2000; Molento et al., 2008; Braga et al., 2009). It is essential that owners, managers and veterinary practitioners take an active and leading role in planning and monitoring effective and appropriate worm control programs for horses, especially based on periodic monitoring and resistance tests (*in vivo* or/and *in vitro*). The appropriate understanding of the resistance status of a worm population also allows the appropriate rotation of anthelmintic classes. The rotation of anthelmintics should be performed when BZ and/or THP are still effective in a property, in order to reduce the use of ML and the selection pressure in parasitic populations. Indeed, all drugs which result not to be effective against small strongyles at the periodic FECRT should be excluded by the rotation. This is especially relevant in cases of multiple resistance.

Another key point that support control measures against anthelmintic resistance in horse small strongyles from Europe, would be the better understanding of the distribution of resistance in Eastern European countries, where, apart the survey recently carried out in

Ukraine (Kuzmina and Kharchenko, 2008) and Slovakia (Cernanska et al., 2009), the information are scattered and lacking. This becomes more important as horse movement between Western and Eastern Europe continues to increase.

Acknowledgments

The author thanks Dr. Albert Boeckh (Pfizer Animal Health, USA) for his valuable comments to the manuscript.

References

- Bjorn H., Sommer C., Schougard H., Henriksen S.A., Nansen P. 1991. Resistance to benzimidazole anthelmintics in small strongyles (Cyathostominae) of horses in Denmark. *Acta Vet. Scand.* 32:253-260.
- Braga F.R., Araújo J.V., Silva A.R., Araujo J.M., Carvalho R.O., Tavela A.O., Campos A.K., Carvalho G.R. 2009. Biological control of horse cyathostomin (Nematoda: Cyathostominae) using the nematophagous fungus *Duddingtonia flagrans* in tropical Southeastern Brazil. *Vet. Parasitol.* 163:335-340.
- Cerňanská D., Paoletti B., Kráľová-Hromadová I., Iorio R., Cudeková P., Milillo P., Traversa D. 2009. Application of a Reverse Line Blot hybridisation assay for the species-specific identification of cyathostomins (Nematoda, Strongylida) from benzimidazole-treated horses in the Slovak Republic. *Vet. Parasitol.* 160:171-174.
- Cernea M.S., Cernea L.C., Cozma V., Raileanu Ş., Gut, A., Silberg R., Madeira de Carvalho L. 2008. Comparative evaluation of drug resistance tests in equine strongylidosis. *Proceedings of the International Equine Parasite Drug Resistance Workshop, Copenhagen, Denmark, 31.7-1.8.2008*, pp. 18-19.
- Chapman M.R., French D.D., Monahan C.M., Klei T.R. 1996. Identification and characterization of a pyrantel pamoate resistant cyathostome population. *Vet. Parasitol.* 66:205-212.
- Coles G.C., Eysker M., Hodgkinson J., Matthews J.B., Kaplan R.M., Klei T.R., Sangster N.C. 2003. Anthelmintic resistance and use of anthelmintics in horses. *Vet. Rec.* 153:636.
- Comer K.C., Hillyer M.H., Coles G.C. 2006. Anthelmintic use and resistance on thoroughbred training yards in the UK. *Vet. Rec.* 158:596-598.
- Craven J., Bjorn H., Henriksen S.A., Nansen P., Larsen M., Lendal S. 1998. Survey of anthelmintic resistance on Danish horse farms, using 5 different methods of calculating faecal egg count reduction. *Equine Vet. J.* 30:289-293.
- Eysker M., Boersema J.H., Kooyman F.N. 1989. Emergence from inhibited development of cyathostome larvae in ponies following failure to remove them by repeated treatments with benzimidazole compounds. *Vet. Parasitol.* 34:87-93.
- Eysker M., Boersema J.H., Kooyman F.N. 1990. Seasonally inhibited development of cyathostomine nematodes in Shetland ponies in The Netherlands. *Vet. Parasitol.* 36:259-264.
- Fisher M.A., Jacobs D.E., Grimshaw W.T., Gibbons L.M. 1992. Prevalence of benzimidazole-resistance in equine cyathostome populations in South East England. *Vet. Rec.* 130:315-318.
- Genchi C., Di Sacco B., Traldi G., Bogara B., Quintavalla F. 1992. Prime osservazioni in Italia sulla resistenza dei piccoli strongili del cavallo (Cyathostominae) ai benzimidazolici ed efficacia del pyrantel pamoato. *Ippologia* 3:77-80. [In Italian].
- Giles C.J., Urquhart K.A., Longstaffe J.A. 1985. Larval cyathostomiasis (immature *Trichonema*-induced enteropathy): a report of 15 clinical cases. *Equine Vet. J.* 17:196-201.
- Harder A., Schmitt-Wrede H.P., Krucken J., Marinovski P., Wunderlich F., Willson J., Amliwala K., Holden-Dye L., Walker R. 2003. Cyclooctadepsipeptides - an anthelmintically active class of compounds exhibiting a novel mode of action. *Int. J. Antimicrob. Ag.* 22:318-331.
- Ihler C.F. 1995. A field survey on anthelmintic resistance in equine small strongyles in Norway. *Acta Vet. Scand.* 36:135-143.
- Kaminsky R., Ducray P., Jung M., Clover R., Rufener L., Bouvier J., Weber S.S., Wenger A., Wieland-Berghausen S., Goebel T., Gauvry N., Pautrat F., Skripsky T., Froelich O., Komoin-Oka C., Westlund B., Sluder A., Mäser P. 2008. A new class of anthelmintics effective against drug-resistant nematodes. *Nature* 452:176-180.
- Kaplan R.M. 2004. Drug resistance in nematodes of veterinary importance: a status report. *Trends Parasitol.* 20:477-481.
- Kaplan R.M., Klei T.R., Lyons E.T., Lester G., Courtney C.H., French D.D., Tolliver S.C., Vidyashankar A.N., Zhao Y. 2004. Prevalence of anthelmintic resistant cyathostomes on horse farms. *J. Am. Vet. Med. Assoc.* 225:903-910.
- Kuzmina T.A., Kharchenko V.A. 2008. Anthelmintic resistance in cyathostomins of brood horses in Ukraine and influence of anthelmintic treatments on strongylid community structure. *Vet. Parasitol.* 154:277-288.

- Lloyd S., Smith J., Connan R.M., Hatcher M.A., Hedges T.R., Humphrey D.J., Jones A.C. 2000. Parasite control methods used by horse owners: factors predisposing to the development of anthelmintic resistance in nematodes. *Vet. Rec.* 146:487-492.
- Love S., McKeand J.B. 1997. Cyathostominosis: practical issue of treatment and control. *Equine Vet. Educ.* 9:253-256.
- Lyons E.T., Tolliver S., Drudge J. 1999. Historical perspective of cyathostomes: prevalence, treatment and control programs. *Vet. Parasitol.* 85:97-112.
- Mair T.S., Sutton D.G., Love S. 2000. Caecocolic and caecocolic intussusceptions associated with larval cyathostominosis in four young horses. *Equine Vet. J.* 32:77-80.
- Meier A., Hertzberg H. 2005. Equine strongyles. II. Occurrence of anthelmintic resistance in Switzerland. *Schweiz. Arch. Tierh.* 147:389-396. [In German].
- Molento M.B., Antunes J., Bentes R.N., Coles G.C. 2008. Anthelmintic resistant nematodes in Brazilian horses. *Vet. Rec.* 162:384-385.
- Murphy D., Love S. 1997. The pathogenic effects of experimental cyathostome infections in ponies. *Vet. Parasitol.* 70:99-110.
- Nilsson O., Lindholm A., Christensson D. 1989. A field evaluation of anthelmintics in horses in Sweden. *Vet. Parasitol.* 32:163-171.
- Osterman Lind E., Kuzmina T., Ugglå A., Waller P.J., Höglund J. 2007. A field study on the effect of some anthelmintics on cyathostomins of horses in Sweden. *Vet. Res. Commun.* 31:53-65.
- Traversa D., Klei T.R., Iorio R., Paoletti B., Lia R.P., Otranto D., Sparagano O.A., Giangaspero A. 2007. Occurrence of anthelmintic resistant equine cyathostome populations in Central and Southern Italy. *Prev. Vet. Med.* 82:314-320.
- Traversa D., von Samson-Himmelstjerna G., Demeler J., Milillo P., Schürmann S., Barnes H., Otranto D., Perrucci S., Frangipane di Regalbono A., Beraldo P., Boeckh A., Cobb R. 2009. Anthelmintic resistance in cyathostomin populations from horse yards in Italy, UK and Germany. *Parasites Vectors*, 2(Suppl 2):S2.
- Uhlinger C.A. 1991. Equine small strongyles: epidemiology, pathology, and control. *Comp. Cont. Educ. Pract.* 13:863-869.
- Varady M., Konigova A., Corba J. 2000. Benzimidazole resistance in equine cyathostomes in Slovakia. *Vet. Parasitol.* 94:67-74.
- von Samson-Himmelstjerna G., Fritzen B., Demeler J., Schürmann S., Rohn K., Schnieder T., Epe C. 2007. Cases of reduced cyathostomin egg-reappearance period and failure of *Parascaris equorum* egg count reduction following ivermectin treatment as well as survey on pyrantel efficacy on German horse farms. *Vet. Parasitol.* 144:74-80.
- Waller P.J. 1987. Anthelmintic resistance and the future for roundworm control. *Vet. Parasitol.* 25:177-191.
- Wirtherle N., Schnieder T., von Samson-Himmelstjerna G. 2004. Prevalence of benzimidazole resistance on horse farms in Germany. *Vet. Rec.* 154:39-41.
- Zinser E.W., Wolf M.L., Alexander-Bowman S.J., Thomas E.M., Davis J.P., Groppi V.E., Lee B.H., Thompson D.P., Geary T.G. 2002. Anthelmintic paraherquamides are cholinergic antagonists in gastrointestinal nematodes and mammals. *J. Vet. Pharmacol. Ther.* 25:241-250.