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Short communication

Use of a reverse line blot assay to survey small strongyle (Strongylida: Cyathostominae) populations in horses before and after treatment with ivermectin

Mariana Ionita [a,*], Daniel K. Howe [b], Eugene T. Lyons [b], Sharon C. Tolliver [b], Ray M. Kaplan [c], Ioan Liviu Mitrea [a], Michelle Yeargan [b]

[a] Department of Parasitology and Parasitic Disease and Animal Biology, Faculty of Veterinary Medicine of Bucharest, Splaiul Independentei nr. 105, Sector 5, 050097 Bucharest, Romania
[b] Department of Veterinary Science, Gluck Equine Research Center, University of Kentucky, Lexington, KY 40546-0099, USA
[c] Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens, GA, USA

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ABSTRACT

A sensitive and specific PCR hybridization assay was applied for species-specific monitoring of the small strongyle (Strongylida: Cyathostominae) populations in horses in a herd before and after treatment with the anthelmintic drug ivermectin. Fecal samples were collected pre- and post-treatment weekly from eight individual horses (four foals and four yearlings) for 6 weeks to determine counts of strongyle eggs per gram of feces (EPGs). Additionally, one foal and one yearling were nontreated controls. Also, one horse, from another herd known to be infected with *Strongylus* spp., was a positive control for these parasites. Genomic DNA was obtained from eggs in groups of approximately 6000–7000 eggs except for two samples containing low EPGs in which 450 eggs were used. Amplification of the intergenic spacers (IGSs) of ribosomal DNA (rDNA) of small and large strongyles followed by reverse line blot (RLB) assay were performed to identify the presence of the 12 most common equine small strongyle species and to discriminate them from *Strongylus* spp. Overall, 11 small strongyle species were identified in pretreatment samples. In the samples collected at 4 weeks after ivermectin treatment, eight small strongyle species were identified and four of them were predominant (*Cylicocyclus nassatus*, *Cylicostephanus longibursatus*, *Cylicostephanus calicatus* and *Cylicostephanus minutus*). At 5 and 6 weeks post-treatment, the RLB assay analysis showed almost the same composition in the small strongyle population as before treatment. *Strongylus* spp. were identified only in samples collected from the positive control horse for these parasites. These data confirm the ability of the PCR-RLB technique for simultaneous species-specific differentiation of equine strongyle eggs, indicating a valuable way of furthering drug-resistance studies.

1. Introduction

The strongyles, as a group, comprise almost one-half of the over 100 species of internal parasites found in horses (Krecek et al., 1987). The small strongyles (Cyathostominae, order Strongylida), also called cyathostomins or cyathostomes, are among some of the most important intestinal nematodes of horses (Lyons et al., 1999) with virtually 100% of horses infected with at least some species of cyathostomins (Reinemeyer et al., 1984). Infection with cyathostomins is complex and produces an inflammatory enteropathy which results in impaired intestinal microcirculation and motility (Love et al., 1999). Clinically, infection with adult cyathostomins can cause mild disease

* Corresponding author. Tel.: +40 740 819 752.
E-mail address: ionitamvet@yahoo.com (M. Ionita).

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symptoms such as intermittent diarrhea, weight loss, poor hair coat, loss of appetite, lethargy with disordered intestinal motility, deterioration of condition, and peripheral edema (Love and Duncan, 1992; Love et al., 1992; Matthews and Morris, 1995). Larval cyathostominosis is characterized by severe diarrhea, protein loss, enteropathy, and weight loss, especially as a result of emergence of massive numbers of larvae from the lining of the large intestines (Love et al., 1999).

The current method of identifying cyathostomin species involves morphological examination of adult stages necessitating the sacrifice of infected horses. Furthermore, species identification of eggs morphologically is not possible and the identification of larvae is difficult and time-consuming using morphological parameters (Gasser et al., 2004). However, recent studies are using genetic markers to accomplish molecular identification. The 26S–18S ribosomal DNA (rDNA) intergenic spacer (IGS) region was chosen as a molecular marker for a comparative sequence analysis of 16 cyathostomin species (Kaye et al., 1998). Sequences across this region showed an interspecific variation between 3 and 60%, indicating that this region may be useful for species differentiation. Based upon these differences, Hodgkinson et al. (2001) have designed four species-specific oligoprobes for identification of the common cyathostomin species *Cylicocyclus* (*Cyc.*) *ashworthi*, *Cylicocyclus* (*Cyc.*) *nassatus*, *Cylicostephanus* (*Cys.*) *longibursatus*, *Cylicostephanus* (*Cys.*) *goldi*, and a fifth oligoprobe to identify all members of this tribe (Hodgkinson et al., 2001).

Recently, a reverse line blot (RLB) assay was developed to identify 13 common species of equine small strongyles simultaneously and to discriminate them from three *Strongylus* spp. (large strongyles) (Traversa et al., 2007). This RLB assay was the basis for our study described herein, which is aimed at species-specific identification of strongyle eggs for monitoring populations of these parasites in horses before and after treatment with the anthelmintic drug, ivermectin.

2. Materials and methods

2.1. Animals

The study population was composed of horses in a herd known to be infected with only small strongyles from a farm in Central Kentucky (Lyons et al., 2008, 2009). Horses in this herd had been treated exclusively with ivermectin approximately four times a year since 1990. Occasionally, the foals were given fenbendazole, oxibendazole, and pyrantel pamoate in addition to ivermectin several times before the present study.

Ivermectin (Zimectrin or Eqvalan, Merial, Duluth, GA, USA) paste was administrated intraorally to each of eight horses (four foals and four yearlings) at 200 μg/kg (Lyons et al., 2008) (Table 1). The body weight of the horses was determined using a girth tape. Additionally, one foal (No. 5) and one yearling (No. 10) were nontreated controls. Also, one horse (No. 11), from another herd known to be infected with *Strongylus* spp., was included as a positive control for these parasites. Fecal samples were collected before and after treatment at different times (Table 1). At each collection, the counts of eggs per gram of feces (EPGs) were determined using the modified Stoll method (Drudge et al., 1963). Fecal cultures (Drudge et al., 1963) were done from feces of the nontreated yearling to confirm the absence of *Strongylus* spp. in the herd.

2.2. Egg isolation

Strongyle eggs were isolated from all fecal samples with positive fecal egg counts. Egg isolation was made using the protocol described by Hodgkinson et al. (2005). Harvested eggs were stored in a small volume of water at −20 °C.

2.3. DNA extraction from eggs

Genomic DNA was isolated from groups of approximately 6000–7000 eggs isolated from individual samples.

Table 1
Data on counts of strongyle eggs per gram of feces (EPGs) in evaluation of effect of ivermectin (200 μg/kg) paste formulation in horse foals born in 2007 and yearlings on a farm in central Kentucky.

Foal No.	Strongyle eggs per gram of feces (EPGs)						
	Day 0	Day 6	Day 20	Day 22	Day 27	Day 34	Day 41
Group I: Treated							
1	2820	0	0	0	0	130	510
2	1750	0	0	0	0	80	180
3	400	0	0	0	0	10	50
4	3410	0	0	0	0	10	80
Group II: Nontreated							
5	750	800	1180	950	2250	1430	350
Yearling No.	Day 0	Day 21	Day 23	Day 25	Day 28	Day 35	Day 42
Group I: Treated							
6	790	0	0	0	30	590	1250
7	960	0	0	0	0	100	710
8	270	0	0	0	10	160	210
9	940	0	0	0	0	330	640
Group II: Nontreated							
10	800	1280	2070	1490	1200	2700	1010