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COPROLOGICAL PREVALENCE OF INTESTINAL PARASITES AND STRONGYLE EPG PROFILES OF WORKING HORSES FROM NORTH-EASTERN AND SOUTH-EASTERN ROMANIA

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Abstract

A coprological study was performed in working horses (n=148) from 13 villages in northeastern and southeastern Romania. The aim of this research was to obtain current data on the prevalence of intestinal parasites in working horses and, additionally, to establish strongyle EPG profiles for the horses based on the strongyle eggs per gram of feces (EPG) counts. For this, fresh fecal samples, collected over a 5-months (June-October) period in 2013, were analyzed qualitatively for presence of intestinal parasites using sodium chloride flotation technique, and quantitatively, for strongyle EPG using a modified McMaster egg counting technique. Fecal samples of 104 horses (70.3%) were positive for parasite eggs, with an overall prevalence as follows: 70.3% for strongyles, 12.2% for Parascaris equorum, 4.1% for Strongyloides westeri, and 2.7% for Anoplocephalidae. The highest intensity rate belonged to strongyles, with the EPG counts varying from 25 to 2775. Of them, 58.6% had the EPG count <250, 23.1% between 250-1000, while for 10.6% of the positive animals the EPG counts ranged between 1000–2000, and for 7.7% was bigger than 2000. The average (%) of EPG-positive animals by age group was: <1year (5.8%), 1-5 (20.2%), 6–10 (29.8%), 11–15 (32.6%), and >16 years (11.5%). This research showed the value of strongyle EPG profiling for the working horses, important base for further studies in designing and monitoring sustainable control program of equine parasites.

Key words: *intestinal parasites, strongyles, working horses, Romania.*

INTRODUCTION

Horses are hosts to a variety of internal parasites. Some of these parasites, usually depending on their abundance, are known to cause problems ranging from reduced performance and condition up to abdominal disease such as colic or severe diarrhoea (Krecek et al, 1987; Love et al., 1999; Mitrea, 2011). The most important parasite group is the cyathostomins, which today consists of more than 50 identified species (Lichtenfels et al., 1998, 2002). Cyathostomins often comprise 95-100% of the total worm burden (Lyons et al., 1999; Wood et al., 2013). Depending on age, the remainder of the worm burden is dominated by species such as *Parascaris equorum*, *Strongyloides westeri*, *Oxyuris equi* and the large strongyle species: *Strongylus vulgaris, S. edentatus, S. equinus*. In addition, tapeworm species, especially *Anoplocephala perfoliata* have been reported to be very common (Gasser et al., 2005).
In the last years, it is well accepted that the assessment of helminth distribution patterns in managed equine populations will yield useful information for developing improved control methods that are less reliant on chemical compounds (Ionita et al., 2010; Nielsen, 2012).
Since the 1960s, numerous studies have reported on increasing levels of cyathostomin resistance to a variety of anthelmintic drugs.
The macrocyclic lactones (avermectin / milbemycins) are currently the only fully effective anthelmintic group (reviewed by Kaplan, 2002, 2004). However, it is widely accepted that avermectin / milbemycin resistance in cyathostomins is inevitable (Lloyd and Soulsby, 1998; Sangster, 1999; Kaplan, 2004). Thus, there is a strong need to revise current approaches to parasite control in order to delay the development of resistance as much as possible.
It is widely accepted that due consideration of the role of parasite *refugia* is key to preserving the efficacy of anthelmintic drugs in worm control programs (van Wyk, 2001; Pomroy, 2006). One way to maximize *refugia* is by

applying selective, targeted treatment as part of a sustainable equine nematode control program (Matthews, 2008; Nielsen, 2012).

One of the basic principles of selective anthelmintic treatment is a consistency of the relative magnitude of strongyle FECs of individual horses over time (Duncan and Love, 1991). Identification of high egg shedders within the herd is an essential goal, and the consistency of egg shedding patterns can be exploited to reduce the number of faecal samples (Gomez and Georgi, 1991; Nielsen et al., 2006; Eysker et al., 2008). With this respect, there are some data to show that strongyle eggs per gram of feces (EPG) profiles can be established for equids (Osterman Lind et al. 1999; Döpfer et al. 2004; Çirak et al. 2005; Lyons et al., 2012).

In Romania, despite of the importance of the horse in various activities (in agriculture, or sport), knowledge and research interests in equine parasites is sparse and fragmentary. Several studies performed in some areas in western, central or eastern Romania, provide only limited data on the prevalence of parasites in horses in Romania. Therefore, the aim of the current study was to acquire further information on the prevalence of intestinal parasites in working horses on several villages in north-eastern and south-eastern Romania and, additionally, to establish strongyle EPG profiles for the horses based on the strongyle eggs per gram of feces (EPG) counts.

MATERIALS AND METHODS

A coprological study was performed in 148 working horses from 13 villages in northeastern and southeastern Romania. All these horses had access to pasture grazing during the study. The animals were assigned in age and gender groups, as follows: foals (up to 1 year, n = 6), yearlings (1 – 5 years; n = 27), and adults of: 6 – 10 years (n = 50), 11 – 15 years (n = 47), 16 – 20 years (n = 16), >20 years (n = 2). Of the total 142 yearlings and adults, 69 were males and 73 were females (Table 1).

Fresh fecal samples, collected over a 5-months (June-October) period in 2013, were analyzed qualitatively for presence of intestinal parasites using sodium chloride flotation technique. Additionally, samples were analyzed quantitatively for strongyle fecal worm eggs counts (FWECs), described as the number of eggs per gramme (EPG) of feces, using a modified McMaster egg counting technique.

Analysis of distribution of working horses with positive strongyle EPG counts by classes of intensity (< 250, 250 – 1000, 1000 – 2000, and > 2000) was undertaken to help comprise the profile pattern.

The statistical analysis was performed using Quantitatively Parasitology 3.0 free software. P values by Fisher's exact test and Chi-square test were computed. $P \leq 0.05$ was considered as statistically significant.

Table 1. **Animals included in the study, stratified by their provenance and age groups**

Region / county[*] (village)	**Age and gender category**											***Total***
	foals <1year	1 – 5 years		6 – 10 years		11-15 years		16 – 20 years		>20 years		
		M	F	M	F	M	F	M	F	M	F	
North-eastern												***93***
SV(Vm)	0	2	2	1	4	2	1	2	1	0	0	*15*
SV (V)	2	0	0	0	6	3	5	0	1	0	0	*17*
IS (C)	1	2	0	4	1	3	3	1	0	0	0	*15*
IS (S,M)	0	3	4	1	1	1	1	2	4	0	1	*18*
NT (T)	0	1	1	6	3	5	0	0	0	1	0	*17*
VN (R.A)	1	2	0	2	3	1	2	0	0	0	0	*11*
South-eastern												***55***
BZ (P.S.V)	0	2	4	3	6	6	2	2	0	0	0	*25*
IL (B.R)	2	1	3	2	7	7	5	1	2	0	0	*30*
Total		***13***	***14***	***19***	***31***	***28***	***19***	***8***	***8***	***1***	***1***	***148***
	6	***27***		***50***		***47***		***16***		***2***		

[*]Counties included in the study: SV – Suceava; IS – Iasi; NT – Neamt; VN – Vrancea; BZ – Buzau; IL - Ialomita

RESULTS AND DISCUSSIONS

Of the 148 fecal samples analyzed, 104 (70.3%) were positive for parasite eggs. Overall, the most prevalent infection was with strongyles (70.3%), followed by *Parascaris equorum* (12.2%), *Strongyloides westeri* (4.1%), and tapeworms - *Anoplocephalidae* (2.7%). Strongyles, *P. equorum, S. westeri* and tapeworm spp. infections were detected on 13 (100%), 10 (76.92%), 7 (53.85%) and 3 (23.08%) of villages, respectively (Table 2).

The highest mean prevalence of strongyles was detected in foals (6/6; 100%), yearlings (20/27; 74.1%) and horses of the 16 -20 age group (12/16; 75.0%). *P. equorum* was detected mainly in foals (5/6; 83.33%) and animals aging over 16 years (5/18; 27.77%) (Table 3).

Table 2. **Prevalence of intestinal parasites in working horses from 13 villages in northeastern and southeastern Romania, according to the age groups of animals**

Parasite species/ Region / county - village	**No. positive (%) / sampled by age**											***Total*** *(%)*
	foals <1year	1 – 5 years		6 – 10 years		11-15 years		16 – 20 years		>20 years		
		M	F	M	F	M	F	M	F	M	F	
Strongyles												
North-eastern												
SV- Vm.	0	2/2	0/2	0/1	3/4	0/2	1/1	2/2	1/1	0	0	*9/15*
SV- V.	2/2	0	0	0	5/6	1/3	4/5	0	0/1	0	0	*12/17*
IS - C.	1/1	2/2	0	4/4	1/1	3/3	3/3	1/1	0	0	0	*15/15*
IS - S.M	0	3/3	3/4	0/1	0/1	0/1	1/1	2/2	3/4	0	1/1	*13/18*
NT - T.	0	0/1	1/1	5/6	2/3	3/5	0	0	0	0/1	0	*11/17*
VN - R.A.	1/1	2/2	0	2/2	3/3	1/1	2/2	0	0	0	0	*11/11*
	4/4	*9/10*	*4/7*	*11/14*	*14/18*	*8/15*	*11/12*	*5/5*	*4/6*	*0/1*	*1/1*	*71/93 (76.3%)*
South-eastern												
BZ – P,S,V	0	2/2	1/4	1/3	0/6	3/6	1/2	0/2	0	0	0	*8/25*
IL – B,R.	2/2	1/1	3/3	0/2	5/7	7/7	4/5	1/1	2/2	0	0	*25/30*
	2/2	*2/3*	*4/7*	*1/5*	*5/13*	*10/13*	*4/7*	*1/3*	*2/2*	*0*	*0*	*33/55 (60.0%)*
Total	6/6	12/13	8/14	12/19	19/31	18/28	16/19	6/8	6/8	0/1	1/1	***104/148 (70.3%)***
Parascaris equorum												
North-eastern												
SV- Vm.	-	1/2	0/2	0/1	0/4	1/2	0/1	2/2	0/1	0	0	*4/14*
SV- V.	1/2	-	-	-	0/6	0/3	1/5	-	0/1	-	-	*2/17*
IS - C.	1/1	0/2	-	0/4	0/1	0/3	0/3	0/1	-	-	-	*1/15*
IS - S.M	-	0/3	0/4	0/1	0/1	0/1	0/1	0/2	0/4	-	0/1	*0/18*
NT - T.	-	0/1	0/1	0/6	0/3	0/5	-	-	-	0/1	-	*0/17*
VN - R.A.	1/1	0/2	-	0/2	0/3	0/1	1/2	-	-	-	-	*2/11*
	3/4	*1/10*	*0/7*	*0/14*	*0/18*	*1/15*	*1/12*	*2/5*	*0/6*	*0/1*	*1/1*	*9/93 (10.7%)*
South-eastern												
BZ – P,S,V	-	1/2	0/4	2/3	2/6	0/6	0/2	2/2	-	-	-	*7/25*
IL – B,R.	2/2	0/1	0/3	0/2	0/7	0/7	0/5	0/1	0/2	-	-	*2/30*
	2/2	*1/3*	*0/7*	*2/5*	*2/13*	*0/13*	*0/7*	*2/3*	*0/2*	*0*	*0*	*9/55 (6.4%)*
Total	5/6	2/13	0/14	2/19	2/31	1/28	1/19	4/8	0/8	0/1	1/1	***18/148 (12.2%)***
Strongyloides westeri												
North-eastern												
SV- Vm.	-	1/2	0/2	1/1	0/4	0/2	0/1	0/2	0/1	-	-	*2/14*
SV- V.	0/2	-	-	-	0/6	0/3	5	-	0/1	-	-	*0/17*
IS - C.	0/1	0/2	-	0/4	0/1	0/3	3	0/1	-	-	-	*0/15*
IS - S.M	-	0/3	1/4	0/1	0/1	0/1	1	0/2	0/4	-	0/1	*1/18*
NT – T.	-	0/1	0/1	0/6	0/3	0/5	-	-	-	0/1	-	*0/17*
VN – R.A.	0/1	1/2	-	0/2	0/3	0/1	2	-	-	-	-	*1/11*
	0/4	*2/10*	*1/7*	*1/14*	*0/18*	*0/15*	*0/12*	*0/5*	*0/6*	*0/1*	*0/1*	*4/93 (4.3%)*
South-eastern												
BZ – P,S,V	-	0/2	0/4	0/3	0/6	0/6	0/2	0/2	-	-	-	*0/25*
IL – B,R.	1/2	0/1	1/3	0/2	0/7	0/7	0/5	0/1	0/2	-	-	*2/30*
	1/2	*0/3*	*1/7*	*0/5*	*0/13*	*0/13*	*0/7*	*0/3*	*0/2*	-	-	*2/55 (3.6%)*
Total	1/6	2/13	2/14	1/19	0/31	0/28	0/19	0/8	0/8	0/1	0/1	***6/148 (4.1%)***
Anoplocephalidae												
North-eastern	0/4	0/10	0/7	0/14	0/18	0/15	0/12	0/5	0/6	0/1	0/1	0/93 (0%)
South-eastern												
BZ – P.S.V.	-	0/2	0/4	2/3	2/6	2/6	0/2	2/2	-	-	-	*4/25*
IL – B.R.	0/2	0/1	0/3	0/2	0/7	0/7	0/5	0/1	0/2	-	-	0/30
	0/2	*0/3*	*0/7*	*1/5*	*2/13*	*1/13*	*0/7*	*0/3*	*0/2*	-	-	*4/55 (7.3%)*
Total	0/2	0/13	0/14	1/19	2/31	1/28	0/19	0/8	0/8	-	-	***4/148 (2.7%)***

Table 3. **Summarized data on number of positive animals for intestinal parasites stratified by age groups**

Parasite species	No. positive (%) / sampled by age											*Total (%)*
	foals <1year	1 – 5 years		6 – 10 years		11-15 years		16 – 20 years		>20 years		
		M	F	M	F	M	F	M	F	M	F	
Strongyles	*6/6*	*12/13*	*8/14*	*12/19*	*19/31*	*18/28*	*16/19*	*6/8*	*6/8*	*0/1*	*1/1*	
	6/6 (100%)	20/27 (74.1%)		31/50 (62.0%)		34/47 (72.3%)		12/16 (75.0%)		1/2 (50.0%)		***104/148 (70.3%)***
	P = 0.407											
Parascaris equorum	5/6	2/13	0/14	2/19	2/31	1/28	1/19	4/8	0/8	0/1	1/1	
	5/6 (83.3%)	2/27 (7.4%)		4/50 (8.0%)		2/47 (4.3%)		4/16 (25.0%)		1/2 0%		***18/148 12.2%***
	P = 0.000											
Strongyloides westeri	1/6	2/13	2/14	1/19	0/31	0/28	0/19	0/8	0/8	0/1	0/1	
	1/6 (16.7)	4/27 (14.8%)		1/50 (2%)		0/47 (0%)		0/16 (0%)		0/2 (0%)		**6/148 (4.05%)**
	P = 0.018											
Anoplocephalidae	0/2	0/13	0/14	1/19	2/31	1/28	0/19	0/8	0/8	-	-	
	0/6 (0%)	0/27 (0%)		3/50 (6.0%)		1/47 (2.1%)		0/16 (0%)		0/2 (0%)		***4/148 (2.7%)***
	P = 0.721											

Table 4. **Number of animals positive for strongyle EPG counts stratified by class of intensity**

Location	Total number		Total no. (%) with positive EPG counts			
	sampled	positive	< 250	250-1000	1000-2000	> 2000
North-eastern						
SV- Vm.	15	9	*6*	*2*	*1*	*0*
SV- V.	17	12	*9*	*0*	*1*	*2*
IS - C.	15	15	*10*	*2*	*1*	*2*
IS - S.	19	13	*6*	*3*	*4*	*0*
NT - T.	17	11	*10*	*0*	*0*	*1*
VN - R.A.	11	11	*1*	*7*	*1*	*2*
South-eastern						
BZ – P,S,V	25	8	*8*	*0*	*0*	*0*
IL – B,R.	30	25	*11*	*10*	*3*	*1*
Total	***148***	***104***	61 *(58.6%)*	24 *(23.1%)*	11 *(10.6%)*	8 *(7.7%)*

Table 5. **Distribution of working horses with positive strongyle EPG counts according to the age groups**

EPG value	Number of animals positive for strongyle EPG counts grouped by age (%)											Total *(%)*
	foals <1year	1 – 5 years		6 – 10 years		11-15 years		16 - 20		>20 years		
		M	F	M	F	M	F	M	F	M	F	
< 250	2 33.3%	5 41.7%	4 44.4%	8 72.7%	8 42.1%	16 88.9%	11 68.8%	3 50%	3 50%	0	0	**60 *(57.7)***
250 - 1000	3 50%	4 33.3%	1 11.1%	1 9.1%	7 36.8%	1 5.6%	3 18.8%	1 16.7%	3 50%	0	1 100%	**25 *(24.1)***
1000 - 2000	0	1 8.3%	4 44.4%	1 9.1%	1 5.3%	0	2 12.5%	2 33.3%	0	0	0	**11 (*10.5*)**
> 2000	1 16.7%	2 16.7%	0	1 9.1%	3 15.8%	1 5.6%	0	0	0	0	0	***8* (*7.7*)**
	6/6	12/13	9/14	11/19	19/31	18/28	16/19	6/8	6/8	0/1	1/1	104/148

A high intensity rate for strongyles was registered, with the EPG counts varying from 25 to 2775. Of them, 57.7% had the EPG count <250, 24.1% between 250-1000, while for 10.5% of the positive animals the EPG counts ranged between 1000–2000, and for 7.7% was bigger than 2000 (Table 4).
The average (%) of EPG-positive animals by age group was: <1year (5.8%), 1-5 (20.2%), 6–10 (28.8%), 11–15 (32.6%), and >16 years (12.5%). Proportion of strongyle EPG-positive animals, stratified by class of intensity and the age groups are presented in Table 5.
Analysis of distribution of working horses with positive strongyle EPG counts by classes of intensity (< 250, 250 – 1000, 1000 – 2000, and > 2000) was undertaken to help comprise the profile pattern. The mean EPG by year of age was lowest (<250) for males of the 6–10 (72.7%), 11–15 (88.9%), and 16 – 20 (50%) age group. The higher mean EPG values (>250) were registered in foals (66.7%), yearlings (57.1%), and females of the 6 – 10 (57.9%), 16 – 20 (50%) age group, and over 20 years (100%). However, of the strongyle positive animals, seven (7.7%) passed high egg counts, over 2000 EPG; of them three were females (42.9%) of 6 – 10 years of age. The remainders were two yearlings, one foal, and one male of the 6 – 10 age group.
The results of the present survey clearly demonstrate that strongyle infections are highly prevalent in working horses in eastern and southern Romania. These findings are consistent with previous reports in Romania which indicate prevalence rates varying between 80.7%, 87.9%, and 100% (Cernea et al., 2003; Covasa and Miron, 2011; Ionita et al., 2013). A similar study in the UK, based on fecal worm egg count (FWECs) has recently reported a mean prevalence of strongyles, *P. equorum*, tapeworm spp. and *S. westeri* of 56, 9, 4 and 8%, respectively (Relf et al., 2012).
The overall highest prevalence of strongyles (up to 70.3% in our study) is not unexpected. All horses with access to pasture are exposed to strongyle infections. Strongyles are considered the most prevalent parasites in horses, particularly small strongyles (Cyathostominae) in well managed farms (Kaplan, 2004). Today, it is generally accepted that cyathostomins (small strongyles) are the most common parasites in horses and the most prevalent cause of disease, ill-thrift and poor performance.
Animal age, last anthelmintic type administered and management practices (for example, group rotation on grazing) most strongly influence strongyle prevalence and level of egg shedding (Relf et al., 2012). Previous studies indicate that, within populations, a relatively small proportion of individual horses are responsible for excreting the majority of strongyle eggs and that there is an element of consistency in the excretion patterns. It is proposed that by identifying animals regarded as 'high egg shedders', this will enable farms and studs to implement more targeted treatment approaches to helminth control (Nielsen et al., 2006).
The acquisition of information on natural distribution patterns will help in establishing appropriate FWEC thresholds at which horses should be treated with an adulticidal ant-helmintic (commonly quoted as 200 - 250 EPG (Uhlinger, 1993; Kaplan and Nielsen, 2010). Subsequently, the judicious application of targeted treatments has potential to control equine strongyle populations by protecting individual horses from high burdens, whilst promoting refugia for anthelmintic susceptible genotypes (Relf et al., 2012; Becher et al., 2010). In the current dataset, a cut-off value of 250 strongyle EPG would indicate that only 42.3% of the population sampled would have required treatment at the time of sampling.

CONCLUSIONS

The results of the present study provide further evidence that the egg shedding levels are influenced by both the age of the horse and level of pasture hygiene. Moreover, these results confirm the value of strongyle EPG profiling for the working horses, as important base for further studies in designing and monitoring sustainable control program of equine parasites.

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