Cyathostomin faecal egg counts in horse farms from Central Italy

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Summary

Cyathostomins, or 'small strongyles', are the most important equine helminths because of their worldwide distribution, spread of anthelmintic-resistant populations, and pathogenic impact. The so-called 'selective treatment' of those animals exceeding a certain faecal egg count (FEC) has recently been proposed to implement cyathostomin control programmes. The present study evaluated the extent of egg shedding in 475 horses living in 12 farms from 3 regions of Italy. All examined farms and 224 horses (47.6%) were positive for cyathostomins. 138 horses (28.8%) scored positive for cyathostomin FECs with a range of eggs-per-gram of faeces (epg) values of 50-2,150. Further 86 horses (18.1%) were positive only under qualitative microscopy (i.e. < 50 epg). Of the animals with a FEC > 50 epg, 81 (17%) and 57 (12%) showed values of 50-200 and > 200 epg, respectively. The findings from this study demonstrated a reduced presence of high-shedding horses compared to results obtained in previous years in the same study areas, despite overlapping climate features in the previous 5 years. A continuing monitoring of epidemiological and biological features of horse cyathostomin infection is crucial for planning intervention programmes aimed to maintaining animal health and preserving the efficacy of parasiticides.

Uova di cyathostomini nelle feci di cavalli del centro Italia

Parole chiave
Cavallo,
Cyathostomini,
Esame coprologico,
Monitoraggio epidemiologico.

Riassunto

I cyathostomini sono i principali parassiti dei cavalli per la loro diffusione cosmopolita, per la loro capacità di resistere ai trattamenti antiparassitari con molti antielmintici e per il loro impatto sanitario sia allo stadio adulto che a quello larvale. Per implementare i programmi di controllo delle infestazioni da cyathostomini, sono stati proposti i cosiddetti "trattamenti selettivi", che intervengono esclusivamente sugli animali che eccedono una determinata conta di uova fecali (FEC). Il presente lavoro ha valutato l'emissione di uova nelle feci di 475 cavalli di 12 scuderie site in 3 regioni italiane. Tutte le scuderie analizzate e 224 cavalli (47,6%), sono risultati positivi per la presenza di cyathostomini. Centotrentotto cavalli (28,8%) hanno mostrato valori di FEC compresi tra 50 e 2.150 uova per grammo di feci (upg) mentre gli altri 86 animali (18,1%) sono risultati positivi solo alla valutazione qualitativa (*i.e.* < 50 upg). Tra i soggetti con FEC > 50 upg, 81 (17%) mostravano valori compresi tra 50 e 200 upg mentre 57 (12%) superavano 200 upg. Rispetto ai risultati ottenuti in passato nella stessa area di studio, questo lavoro dimostra una riduzione della presenza di cavalli contaminati, a fronte di dati metereologici sovrapponibili nei cinque anni trascorsi dall'ultimo studio. Per pianificare programmi di intervento finalizzati contemporaneamente a conservare la salute dei cavalli e a preservare l'efficacia delle molecole antielmintiche, risulta cruciale il monitoraggio continuo dell'epidemiologia e della biologia delle infestazioni da cyathostomini nei cavalli.

Introduction

Small strongyles or cyathostomins (Nematoda, Strongylidae) are regarded as the most significant horse parasitic helminths (Traversa *et al.* 2009, Kaplan and Nielsen 2010). Virtually 100% of horses may harbour small strongyles, that may infect all age classes of animals (Pickles *et al.* 2010, Fritzen *et al.* 2010). Although high worm burdens do not necessarily lead to clinical disease or to faecal egg excretion, the infection can result in malaise, anorexia, colic, and weight loss (Love *et al.* 1999, Corning 2009). The simultaneous emergence of encysted larvae from the gut wall may induce a syndrome known as 'larval cyathostominosis', which is characterised by weight loss, diarrhoea, colics, ventral oedema, and a high mortality rate (Lyons *et al.* 2000, von Samson-Himmelstjerna 2012).

The control of small strongyles relies on the use of benzimidazoles (BZ), tetrahydropyrimidines (THP), and macrocyclic lactones (ML) (Matthews 2008, Kaplan and Nielsen 2010). It is well-known that cyathostomin are resistant to BZ and THP. Recent studies have additionally proposed an initial resistance to ML, especially ivermectin (Lyons and Tolliver 2013, Geurden *et al.* 2014).

New proposals for the control of horse cyathostomins have focused on preserving the efficacy of parasiticides, and include minimising unnecessary treatments in animals excreting a low number of eggs to increase the proportion of parasites that remain unexposed to anthelmintic (*i.e. refugia*) (van Wyk 2001). These so-called 'selective treatments' aim at keeping parasite burdens below levels that could cause disease, thus simultaneously preserving the efficacy of anthelmintics. The identification of those horses requiring anthelmintic treatment is based on the evaluation of faecal egg counts (FECs). Only animals exceeding a certain threshold should be treated. There is a general consensus for a cut-off is ≥ 200 eggs per gram (epg) of faeces (Matthee and McGeoch 2004, Nielsen *et al.* 2014).

A large-scale study carried out in Italy in 2008 (Traversa *et al.* 2009) demonstrated that the efficacy of BZ was reduced in about one-third of the examined premises, and that the efficacy of THP was reduced in about 20-30% of the examined yards. The results of the study by Traversa and colleagues resulted in the selective treatment strategy being promoted among veterinarians. The same study also demonstrated that the percentage of horses with FECs ≥ 200 epg was relatively high (Traversa *et al.* 2010). However, there are no further studies demonstrating the application of this strategy and thus no updated data on the extent of egg excretion of strongyle eggs in horse farms from Italy. Following the proliferation of scientific information about the resistance to parasiticides in this area during previous years, the present study aims to evaluate cyathostomin occurrence and the FECs trends in farms located in 3 different region of Italy and with a long-lasting history of cyathostomin infection since 2008.

Materials and methods

Farms and animals

From April to July 2011 and from April to September 2012, individual faecal samples were collected from 475 horses living in 12 farms located in the Abruzzo, Lazio, and Tuscany regions of central Italy (Figure 1).

The study sites were selected based on the following criteria:

I. at least 8 permanently resident horses per farm (we selected a range of 8-131 equines per farm);
II. farms with a previous history of cyathostomin infection or located in areas where these parasites are endemic (Traversa *et al.* 2009, Traversa *et al.* 2010, Sconza *et al.* 2013). Our sample included 10 of the 12 farms that had been sampled during previous studies and, according to the owners, fewer than 25% of the horses had changed;
III. anthelmintic treatments had not been administered for at least 4 months prior to testing;

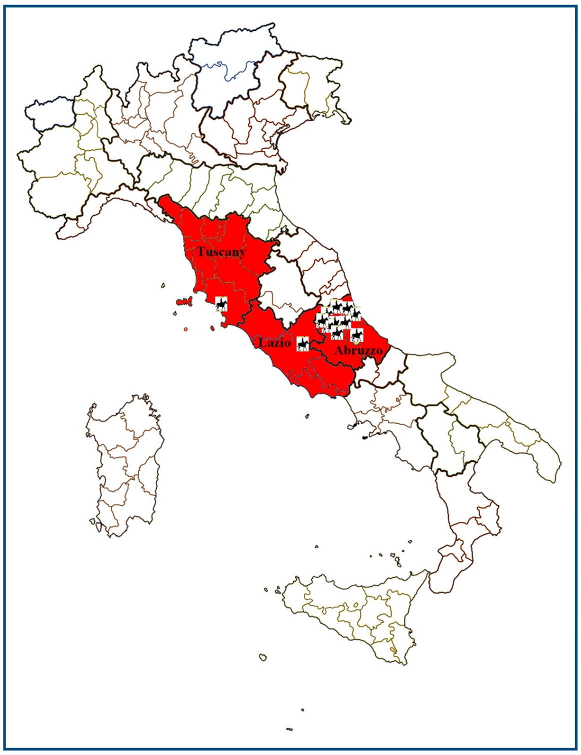

Figure 1. *Farm locations.* Abruzzo, Lazio and Tuscany: geographic distribution of the farms included in the study.

IV. willingness of the owners and managers to participate in the study;

V. a previous history of reduced efficacy of parasiticides and evaluation of FECs in horse operations in the same regions (Traversa *et al.* 2009, Traversa *et al.* 2010);

VI. owners and practitioners who were aware of the possibility of selective treatments.

All horses present in each site were sampled, *i.e.* 279 mares, 65 stallions, and 131 geldings with an age range of between 6 months and 27 years (mean = 7 years). Horses were kept for sporting purposes (*e.g.* jumping, 3-days events, and western shows), pleasure riding, or were retired from previous activities. The majority of horses lived in a box and were allowed into paddocks no more than 3-4 hours a day (351 of 475 horses). The other horses (124) grazed more than 4 hour a day. Faeces were removed regularly.

Copromicroscopic examinations

Individual faecal samples were collected from the rectum of each horse and analysed within 24 hours. Analysis took place at the Faculty of Veterinary Medicine of Teramo, Italy, with a standard flotation technique ($NaNO_3$ solution with a specific gravity of 1,350), and with a modified McMaster method with a sensitivity of 50 epg (Taylor *et al.* 2007).

Up to ~ 100 grams of pooled faeces obtained from samples collected in each farm were mixed with oak sawdust and water and incubated for 10 days at 27 °C and ~ 75% relative humidity. One and 2 pooled cultures were prepared for farms with fewer and more than 15 horses, respectively. After incubation, third-stage larvae (L3) were harvested using baermanisation. Approximately 100 L3s for each sample were examined using a light microscope and identified (MAFF 1986).

Statistical analysis

A descriptive statistical analysis was carried out using Microsoft Excel® (2014). Percentage values were rounded to the nearest integer and climate variables, such as mean, highest, and lowest temperatures; and humidity, rainfall, and wind speed, were collected for the semester prior to the study. These data were differentiated among the 3 enrolled regions and the 3 different study years, and compared using a 1-way variance analysis. The effect of gender on the FECs was analysed using a 1-way ANOVA. To compare the effect of the horse breed on the FECs, 2 categories were created: i) Western show breeds, such as Quarter horse (84 horses), Paint (15 horses), and Appaloosa (20 horses); and ii) Warmblood breeds, such as Italian Warmblood (250 horses), Belgian Warmblood (20 horses), Haflinger (9 horses), Maremmano (4 horses), Andalusian horses (6 horses), and Mixed-breed horses (67). The FECs of these 2 categories were compared using a Mann-Whitney test. An independent sample t-test was performed to evaluate differences in the FECs of horses which mostly lived in boxes and those which grazed. The same analysis was applied to test the effect of horse ages on the FECs using 2 age classes, *i.e.* younger than 2 years and older than 2 years.

All statistical tests were performed with Medcalc® (Version 12.5), with a significance of $P < 0.05$.

Results

All farms (100%) were positive for the presence of cyathostomin eggs. Overall, a 47.6% infection rate was found in the animal populations, with a range of 7.7%-100% in the single study sites (Table I). 138 horses (28.8%) showed cyathostomin FECs ranging from 50 to 2,050 epg. Further 86 horses (18.1%) were positive for up to 50 epg (*i.e.* only at the qualitative examination). Of the animals with

Table I. *Distribution of the faecal egg count (FEC) of 475 horses within 12 farms in Central Italy.*

Farm	H/Y	pos	neg	epg < 50	50 ≤ epg ≤ 200	epg > 200	FEC M (± SD)	A
1	42	28	14	4	15	9	225.60 (± 377.79)	3
2	14	10	4	3	3	4	167.86 (± 239.07)	0
3	21	8	13	1	4	3	183.33 (± 469.93)	2
4	35	17	18	9	5	3	47.14 (± 133.34)	0
5	8	5	3	2	3	0	43.75 (± 67.81)	0
6	36	20	16	7	10	3	58.33 (± 121.01)	1
7	13	11	2	1	3	7	580.77 (± 59.08)	2
8	13	1	12	1	0	0	3.85 (± 13.87)	0
9	43	4	39	1	3	0	5.81 (± 19.55)	2
10	12	12	0	0	3	9	308.33 (± 157.87)	0
11	131	72	59	33	22	17	305.13 (± 416.88)	3
12	107	36	71	24	10	2	137.50 (± 158.29)	3

H/Y = number of horses per yard, pos= number of horses positive for cyathostomins; neg = number of horses negative for cyathostomins; epg = eggs per gram of faeces; M = arithmetic mean; SD = standard deviation; A = number of horses positive for ascarids (*Parascaris equorum*).

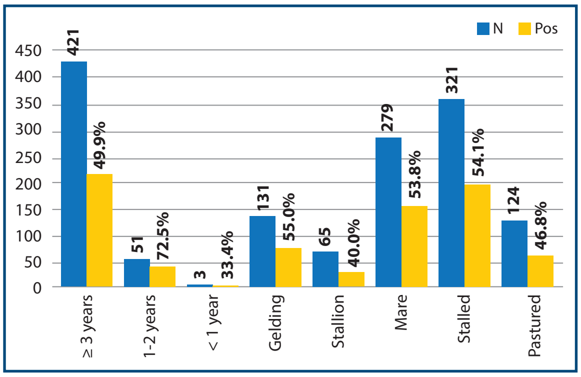

Figure 2. *Positivity for cyathostomins in relation to age, sex and living habits of 475 horses from 12 farms from Italy.*

N = total amount of horse within each category; Pos = positivity within the category; % = positivity percentage in each category.

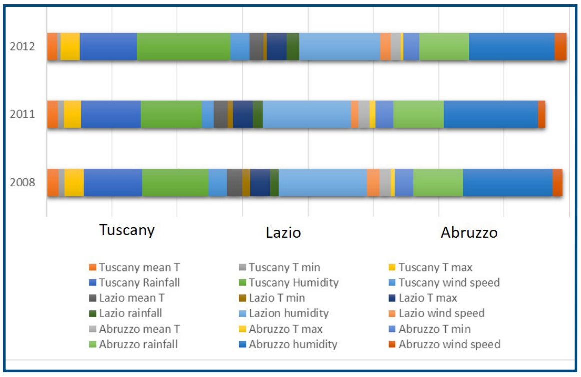

Figure 3. *Graphical representation of the temperature, humidity, rainfall and wind speed trend in Tuscany, Lazio and Abruzzo during the six months prior the studies sample collection (Source: www.ilmeteo.it).*

a FEC > 50 epg, 81 (17%) and 57 (12%) showed epg values of 50-200 and > 200 epg, respectively (Table I). The microscopic examination of the *in vitro* cultured L3s showed that they belonged exclusively to the Cyathostominae subfamily.

Other than occasional findings of strongyloids and pinworms, 16 horses (3.4%) from 7 sites scored positive for *Parascaris equorum* in both single and mixed infections with cyathostomins. Six horses (2.5%) were positive for *P. equorum* with a range of epg values of 50-450, while 10 (2.1%) were positive only at the flotation (*i.e.* epg < 50). Infection prevalence in relation to age, sex, and living habits for Cyathostomins are shown in Figure 2.

Climate variables did not change significantly from 2008 to 2012 in all study regions (Figure 3). The statistical analysis did not show any difference in the distribution of FECs for this study among gender, age, breed, and living habits categories.

Discussion

The data from this study reflect a typical over-dispersion of parasites (Nielsen 2012, Lester and Matthews 2014), where a minority of horses shed most of the eggs in a population (Traversa *et al.* 2010, Hinney *et al.* 2011). In fact many horses harboured low parasite burdens (47.2% of the population had FECs ≤ 200 epg), while others carried more moderate parasite levels, and only few individuals shed the highest levels of faecal eggs (12% of the horses showed FECs > 200 epg). There was no linear correlation between FECs and the intestinal cyathostomin burden – thus high levels of egg shedding are not necessarily equivalent to an increased risk of clinical disease for an individual animal (Duncan and Love 1991, Nielsen *et al.* 2014).

The difference between the results of this study and those obtained in the aforementioned 2008 study, *i.e.* where a percentage of 'high-egg shedders' was higher than that of 'low-egg shedders' (Traversa *et al.* 2010), could be due to a different age pattern of the horses enrolled in the respective studies. Studies showed that the predictive probability for strongyle FEC decreased with age (Nielsen *et al.* 2006) and, accordingly, more than 90% of the horses in this study were ≥ 3 year old. In this study, a small number of horses with a FEC > 200 epg were responsible for 83.3% of the total egg output detected at the copromicroscopic examination. These results are consistent with those of a recent UK survey that showed that only a minority of horses were responsible for excreting the majority of strongyle eggs in 22 premises (Relf *et al.* 2013). In contrast with Relf and colleagues (Relf *et al.* 2013), who found a higher distribution of 'high shedders' among horses younger than 2 years (62.4%) compared to the older animals (37.6%), in this study the majority of horses (87.7%) were older than 3 years. Only few adult horses, *i.e.* 16 (6.4%), scored positive for *P. equorum* eggs and the majority of them (10 of 16) were positive only to the flotation. This finding was likely influenced by the age of the enrolled population, given that horses younger than 3 years were 17.7% of the overall study population, and suggests that most animals were able to develop adequate immunity against this parasitosis.

This study can be considered a basis for further studies investigating the application of selective strategies on a larger scale in Italy. This is noteworthy considering that horses have a strong tendency to remain at the same level of egg shedding over time, *i.e.* 'low-shedding horses' are likely to remain this way regardless of any administered treatment, thus justifying selective treatments only in 'high shedding' populations (Larsen *et al.* 2011, Nielsen 2012). The weather parameters did not significantly change during this study, however they should be taken into account in larger-scale studies because they are reported to influence the development of cyathostomin free-living stages (Corning 2009).

Selective treatments may be effective in reducing pasture contamination and the number of parasiticide administrations, and in preserving cyathostomin *refugia*. Importantly, despite scientific consensus that further studies are necessary to ultimately confirm this possible correlation, previous studies have demonstrated certain reductions of anthelmintic resistance on farms using selective treatments (von Samson-Himmelstjerna 2009, Matthews 2014). The selective treatment strategy requires frequent monitoring of egg excretion, *e.g.* twice a year (Nielsen *et al.* 2006), in order to promptly identify those horses with an egg shedding above the treatment threshold. The application of treatment should moreover be thoroughly evaluated on a case-by-case basis, considering the age classes, living habits, and risk for diseases caused by other parasites, *e.g. P. equorum* or *Strongylus vulgaris*.

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