A survey on Cyathostominae nematodes (Strongylidea, Strongylidae) in pasture bred horses from São Paulo State, Brazil

Nematóides Ciatostomíneos (Strongylidea, Strongylidae) em eqüinos criados a pasto no Estado de São Paulo, Brasil

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Abstract: By previous fecal examinations, 14 naturally nematode infected equine hosts were selected from horse-breeding farms in Jaboticabal County, São Paulo State, Brazil. The animals were divided in two experimental groups according to age: seven animals, 12 to 24 months old, were allotted to the Young Group and the other seven equines, all of them with more than 60 months old, constituted the Adult Group. One week later all 14 equine hosts were slaughtered and the separate contents, plus a thorough scraping, of each section of the gastro-intestinal tract (stomach, small intestine and large intestine), were collected. Nematodes from every segment were identified, sexed and counted, and emphasis was put on the Cyathostominae found in the large intestine. The prevalent Cyathostominae species found were carefully studied.From the total of 537,128 nematodes collected from the 14 animals of both age groups, 80.5% belonged to the Cyathostominae sub-family (small strongyles). From these, 72.64% came from the Young Group hosts and the remaining 27.36% from the Adult one. The hosts of the Young Group harbored 22 species of Cyathostominae, while those of the Adult Group had only 14 species.This study points out the higher prevalence of the Cyathostominae among the nematode parasites of equines from the studied area in Brazil.
Key words: Cyathostomes, horses, age.

Resumo: Foram selecionados, por meio de exames coprológicos, 14 eqüinos naturalmente infectados por nematóides, oriundos de haras do município de Jaboticabal, São Paulo, Brasil. Os animais foram distribuídos em dois grupos experimentais de sete animais cada, conforme a faixa etária: entre 12 e 24 meses (jovens) e com mais de 60 meses de idade (adultos). Após uma semana, os 14 equinos foram necropsiados e todo conteúdo e raspado de cada segmento do trato gastrintestinal (estômago, intestinos delgado e grosso) foram recolhidos. Os nematóides presentes em todos segmentos anatômicos foram identificados, sexados e contados. Foi dada ênfase aos Cyathostominae encontrados no intestino grosso. De um total de 537.128 nematóides recuperados dos 14 animais necropsiados, 80,5% pertenciam à sub-família Cyathostominae (pequenos estrôngilus). Destes, 72,64% foram encontrados nos equinos jovens e os 27,36% restantes nos animais adultos. Os animais jovens apresentaram 22 espécies de Cyathostominae, enquanto os adultos apresentaram apenas 14 espécies. Este estudo aponta a maior prevalência de ciatostomíneos, entre os nematóides parasitos, principalmente em equinos de 12 a 24 meses de idade, oriundos da região estudada.
Palavras-chave: Ciatostomíneos, faixa etária, eqüinos, nematóides.

1 Introduction

Scientific emphasis on the Cyathostominae nematode group the increased in the last 15 years greatly due to the clinical evaluation of parasitism (REINEMEYER, 1986; HERD, 1990). In the last twenty years the search for anthelmintic efficiency of drugs against the Strongylidae as a group has been conducted, but the contemporary tendency is to face the problem at an individual species level (REINEMEYER, 1986).

After outlining the problem of cyathostomiasis, unduly kept, for over half a century in Brazil as a matter of low relevance, this study represents a conscient effort, following the leadership of Lanfredi (1983), to try and restore the scientific priority given to it by Vaz in the decade of 1930-39. Within this spirit, the long term goal of the project is parasite control, mostly of nematodes, in domestic equids. However, as such a control has never been wholly achieved anywhere, its fulfillment may be considered a remote possibility, to be reached through several intermediary steps, one of which is a precise knowledge of the worm species present in each situation.

In this paper are results of a study of taxonomic and quantitative nature, comparing the helminth populations found at *postmortem* examinations of naturally infected horses belonging to two age groups, young and mature animals respectively, all of which were from the Jaboticabal area.

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2 Material and Methods

Through previous fecal egg counts (EPG), 14 equids (*Equus caballus*) of nondescript breed, half of them (the young group) 12 to 24 months of age and the other half more than 60 months old (the adult group), were obtained in Jaboticabal county, State of São Paulo, Brazil. These animals were purchased at random with the only condition being a daily passage of at least 1,000 nematode eggs per gram of feces. After dividing the animals by age they were kept indoors in two independent stalls for one week, after which they were killed in seven successive pairs, always composed of an young and an adult animal, taken at random from their respective age group and necropsied.

Specific diagnosis was made by means of a stereomicroscope equipped with a phase contrasting system and applying the taxonomic criteria published by Lichtenfels (1975). Later on, the taxonomy was put up to date according to Lichtenfels *et al.* (1997).

The Fischer's Exact Test was employed (SAS INSTITUTE, 1989) for the variance analysis of the two treatments (young and adult groups).

3 Results

Helminthologic results according to nematode found in the 14 equine hosts are presented in Tables 1 to 2 and in Figures 1 to 3.

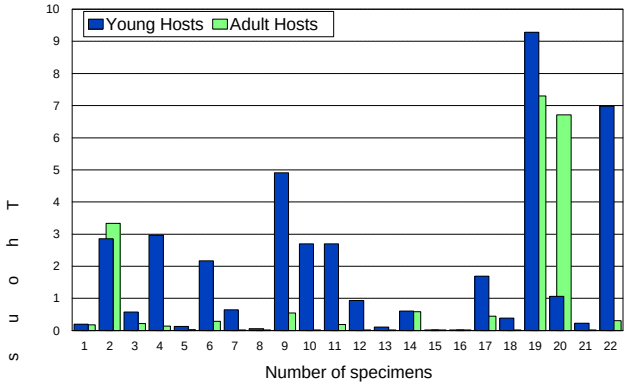

Figure 1 – Arithmetic average specimen whole counts for each of 22 small strongyle species from 14 necropsied groups.

Concerning Cyathostominae[1] (Table 1), 21 species of this sub-family, belonging to eight genera (*Cyathostomum*, *Coronocyclus*, *Petrovinema*, *Cylicocyclus*, *Cylicodontophorus*, *Parapoteriostomum*, *Cylicostephanus* and *Poteriostomum*) were recovered from the 14 necropsies. In the young group (12 to 24 months old) all 21 species were present, while in the mature group (over 60 months old) only 13 of those were found. All six genera were represented in both host groups.

Figure 1 shows the average number (arithmetic mean) of each species of cyathostomine in each group of hosts. The species *Cylicostephanus longibursatus* appeared in all 14 hosts and showed the highest numbers in both groups, even if these did not differ significantly from one another. *Cylicostephanus goldi* was found with second highest numbers and was found infected 12 of the 14 hosts.

The 10 most prevalent species in this study were, in decreasing order: *C. longibursatus*, *C. goldi*, *Cylicocyclus insigne*, *Cylicocyclus nassatus*, *Cyathostomum catinatum*, *Coronocyclus coronatus*, *Cylicocyclus leptostomum*, *Parapoteriostomum euproctus*. *Cylicostephanus minutus* and *Coronocyclus labiatus*.

1. *Cyathostomum pateratum*	9. *Cylicocyclus insigne*	17. *Cylicostephanus minutus*
2. *Cyathostomum catinatum*	10. *Cylicocyclus elongatus*	18. *Cylicostephanus calicatus*
3. *Coronocyclus coronatus*	11. *Cylicocyclus leptostomum*	19. *Cylicostephanus longibursatus*
4. *Coronocyclus labiatus*	12. *Cylicocyclus radiatus*	20. *Cylicostephanus goldi*
5. *Coronocyclus labratus*	13. *Cylicodontophorus bicoronatus*	21.*Poteriostomum imparidentatum*
6. *Cylicocyclus nassatus*	14. *Parapoteriostomum euproctus*	22. Immature forms
7. *Cylicocyclus ashworthi*	15. *Parapoteriostomum mettami*	
8. *Cylicocyclus ultrajectinus*	16. *Petrovinema poculatum*	

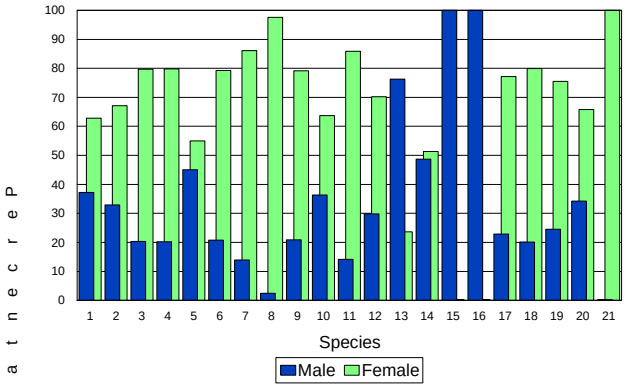

Figure 2 – Male and female mean percent counts of specimens for each of 21 Cyathostominae species found in the young horse groups.

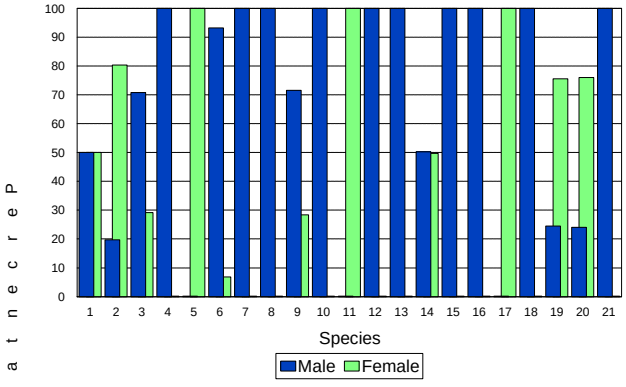

Figure 3 - Male and female mean percent counts of specimens for each of 21 Cyathostominae species found in the adult horse groups.

[1]*Coronocyclus coronatus* (Lichtenfels, 1997) = *Cyathostomum coronatum* (Lichtenfels, 1975)
Coronocyclus labiatus (Lichtenfels, 1997) = *Cyathostomum labiatum* (Lichtenfels, 1975)
Coronocyclus labratus (Lichtenfels, 1997) = *Cyathostomum labratum* (Lichtenfels, 1975)
Parapoteriostomum mettami (Lichtenfels, 1997) = *Cylicodontophorus mettami* (Lichtenfels, 1975)
Parapoteriostomum euproctus (Lichtenfels, 1997) = *Cylicodontophorus euproctus* (Lichtenfels, 1975)
Petrovinema poculatum (Lichtenfels, 1997) = *Cylicostephanus poculatum* (Lichtenfels, 1975)

Nematodes other than Cyathostominae recovered from the hosts were of 10 diferent species (Table 2). In the young group (12 to 24 months old) the only species not was *Crasterostomum acuticaudatum*, while in the mature group (over 60 months old) *Parascaris equorum* and *Strongylus vulgaris* were not present.

The 10 species of nematodes other than Cyathostominae recovered in this study were: *Trichostrongylus axei, Habronema muscae, Parascaris equorum Triodontophorus spp, Strongylus vulgaris, Strongylus edentatus, Gyalocephalus capitatus, Oxyuris equi, Craterostomum acuticaudatum and Anoplocephala perfoliata.*

4 Discussion

The main species of Cyathostominae found in horses from Jaboticabal county were practically the same as found in other countries and the similarity of results of this survey with others show that the prevalence of several of these species remain stable. This suggests that if a change in population levels ocurred it was slight and could be attributed to the advent of modern anthelmintic drugs.

Lanfredi (1983) called attention to disagreements between lists of species prepared by Theiler (1923), Lichtenfels (1975) and Ogbourne (1976), respectively, stating that such discrepancies occur because either some of those species are more common in *Equus asinus*, than in *Equus caballus*, or some species are "rare" or even "very rare" at the areas where most of the surveys were conducted. As an example of such a situation, in this study *Poteriostomum imparidentatum* was detected in animals of the young group, a species also found by Lichtenfels (1975) but not by Lanfredi (1983) or Ogbourne (1976), even when this last author examined 83 equine hosts.It must be emphasized that *P. imparidentatum* was found in only one host of the young group, in a total of 1,600 parasites (less than 1% of the group's parasites burden). In many papers published prior to 1975 a separation of parasites found in *Equus asinus* and *Equus caballus* was not considered. It is still a controversial point if there exits any cross-infection between these two host-species for all or any of their parasitic cyathostomine species (LANFREDI, 1983). Several foreign papers also testify to the importance of the Cyathostominae (HERD, 1990, UHLINGER, 1990, HERD, 1991, MAIR, 1993).

Concerning Cyathostominae genera recognized in the 14 equids used, the results of this study show that some of these species differ statistically in young and adult groups of hosts by the Fischer's Exact Test. These were:*Coronocyclus coronatus, Cylicocyclus ashworthi, Cylicocyclus elongatus, Cylicocyclus leptostomum, Cylicocyclus radiatus, Cylicostephanus minutum* and *Cylicocyclus calicatum* (Table 1). The total counts of Cythostominae specimens (Table 2) showed no significant difference according to statistical analysis between young and adult groups suggesting that these parasites infect their individual hosts when they are young and keep parasitizing them through adult age. Within the young group, the specimen counts of *Cylicocyclus* and *Cylicostephanus* genera represented nearly two thirds (64.29%) of the total number; while in the adult group, the *Cylicostephanus* genus contained more than 70% of the cyathostomine nematode burden. These results seem to follow an ample geographic tendency, as pointed out by several authors (FOSTER,1936, REINEMEYER *et al.*, 1984, LOVE e DUNCAN, 1992, SILVA *et al.*, 1995).

Table 1 – Cyathostominae species and immature form average counts, their individual ranges of variation, number of uninfected hosts, prevalence in young and adult group of horses and statistical significance.

Specie of Cyathostominae	Young Group				Adult Group				Fischer's Exact Test*
	Average counts	Range	Uninfected hosts	Prevalence (%)	Average counts	Range	Uninfected hosts	Prevalence (%)	
Cyathostomum pateratum	193	0 - 598	2	71,42	181	0 - 1269	6	14,28	0,1026
C. catinatum	2857	71 - 6110	1	100,00	3340	0 - 18795	4	42,85	0,0699
Coronocyclus coronatus	569	177 - 1176	0	100,00	220	0 - 786	5	28,57	0,0210
C. labiatus	2964	0 - 10501	1	85,71	133	0 - 753	5	28,57	0,1026
C. labratus	125	0 - 398	3	57,14	27	0 - 192	6	14,28	0,2657
Cylicocyclus nassatus	2171	0 - 10095	1	85,71	289	0 - 773	3	57,14	0,5594
C. ashworthi	648	0 - 1939	2	71,42	0	0 - 0	7	0,00	0,0210
C. ultrajectinus	59	0 - 410	6	14,28	0	0 - 0	7	0,00	1,0000
C. insigne	4906	1576 - 10326	0	100,00	548	0 - 2901	3	57,14	0,1923
C. elongatus	2695	109 - 7213	0	100,00	0	0 - 0	7	0,00	0,0006
C. leptostomum	2693	197 - 10095	0	100,00	186	0 - 788	5	28,57	0,0210
C. radiatus	934	0 - 2972	1	85,71	0	0 - 0	7	0,00	0,0047
Cylicodontophorus bicoronatus	107	0 - 397	5	28,57	0	0 - 0	7	0,00	0,4615
Parapoteriostomum euproctus	601	0 - 2850	2	71,42	582	0 - 3813	4	42,85	0,5921
P. mettami	5	0 - 32	6	14,28	0	0 - 0	7	0,00	1,0000
Petrovinema poculatum	21	0 - 82	5	28,57	0	0 - 0	7	0,00	0,4615
Cylicostephanus minutus	1687	84 - 4981	0	100,00	448	0 - 3135	6	14,28	0,0047
C. calicatus	387	0 - 754	1	85,71	9	0 - 64	6	14,28	0,0291
C. longibursatus	9285	654 - 25651	0	100,00	7296	64 - 19362	0	100,00	1,0000
C. goldi	1067	0 - 4007	2	71,42	6713	77 - 21579	0	100,00	0,4615
Poteriostomum imparidentatum	229	0 - 1061	6	14,28	0	0 - 0	7	0,00	1,0000
Immature forms	6983	383 - 11542	0	100,00	310	0 - 1515	4	42,85	0,0699

* Values lower than 0,05 show significant difference between age groups.

Table 2 – Average parasite counts, their individual ranges of variation, number of uninfected hosts, prevalence in young and adult group of horses and statistical significance.

Specie of Nematode	Studied parameters								Fischer's Exact Test *
	Young group				Adult group				
	Arithmetic mean *	Range	Number of uninfected hosts	Prevalence (%)	Arithmetic mean	Range	Number of uninfected hosts	Prevalence (%)	
Trichostrongylus axei	70	0 - 290	5	28,57	5	0 - 15	6	14,28	1,0000
Habronema muscae	80	0 - 210	1	85,71	5	0 - 15	1	85,71	1,0000
Parascaris equorum	60	0 - 120	2	71,42	0	0 - 0	7	0,00	0,0210
***Triodontophorus* spp**	12435	150 - 27000	0	100,00	115	0 - 790	6	14,28	0,0047
Strongylus vulgaris	110	0 - 75	4	42,85	0	0 - 0	7	0,00	0,1923
Strongylus edentatus	85	0 - 595	6	14,28	10	0 - 40	5	28,57	1,0000
Craterostomum acuticaudatum	0	0 - 0	7	0,00	110	0 - 790	4	42,85	0,1923
Gyalocephalus capitatus	120	0 - 440	5	28,57	155	0 - 515	2	71,42	0,2861
Oxyuris equi	1580	0 - 6330	1	85,71	200	0 - 600	2	71,42	1,0000
Anoplocephala perfoliata	10	0 - 50	5	28,57	5	0 - 15	5	28,57	1,0000
Cyathostominae	41305	20635 - 81665	0	100,00	20440	4280 - 56440	0	100,00	1,0000

* - Values lower than 0,05 show significant difference between age groups.

None of the adult equids was infected with *Cylicocyclus ashworthi, Cylicocyclus ultrajectinus, Cylicocyclus elongatus, Cylicocyclus radiatus, Cylicodontophorus bicoronatus, Cylicostephanus mettami, Petrovinema poculatum* and *Poteriostomum imparidentatum.* (Table 1).

In the total of nematode species found in the 14 killed hosts (Table 2), *Trichostrongylus axei* had a low prevalence and small parasitic burden in both groups. In 1981, Pandey *et al.* reported a prevalence of 80.0% of *T. axei* in equine hosts in Morocco.

None of the adult equids used were infected with *Parascaris equorum*, an ascarid. This confirmed Urquhart *et al.* (1990), who reported prevalence of this species as higher in young and low in adults. The counts of this species for each age group were significantly different according to Fischer's Exact Test.

Strongylus vulgaris was restricted to the young group, with a prevalence of 42.9% (average of 110 parasites per host). Several researchers (REINEMEYER, 1986; UHLINGER, 1990, CHAPMAN, 1991,) believe that the "large strongyles", because of modern anthelmintic drugs, cease to be the most important parasitic nematodes of equid hosts. This applies to *Strongylus edentatus* in this study, since the prevalence was 14.28% and 28.57% respectively for the young and adult groups.

The genus *Triodontophorus* was 100% prevalent (average of 12,435 parasites per host) in the young and 14.28% (average of 115 parasites) in the adult groups, which brought the difference between groups concerning the number of infected horses, to be statistically significant by the Fischer's Exact Test.

Oxyuris equi appeared in both groups with prevalences of 85.71% (average of 1580 parasites) in the young group and 71.42% (average of 200 parasites) in the adult one. All the *O. equi* specimens parasitizing young hosts were mature and those infecting adults were immature ones; this seems to be one of the manners by which age-resistance of hosts against invading organisms manifests itself. This finding is in agreement with previous observations (DRUDGE, 1972).

The cestode, *Anoplocephala perfoliata*, was present in low prevalence (28,57%) in both groups and showed a small parasite burden, being found in only two animals of each group. The difference between groups was not significant though; probably this was due to a sampling deficiency. Reinemeyer *et al.* (1984) link the low prevalence of this cestode species to age-resistance, which would cause adult hosts to have a decreasing propensity to become infected.

The prevalence of *Habronema muscae* was of 85.7% in both groups, but the young ones were nearly 15 times (570 parasites) as infected as the adults (38 parasites).

Craterostomum acuticaudatum was found in only three animals of the adult group (prevalence of 42.85%). Prevalence was too low for a definitive conclusion, since it is improbable that this species is restricted to adult hosts.

Gyalocephalus capitatus, a species whose genus is no more considered part of the group Cyathostominae (HARTWICH, 1986, *apud* LICHTENFELS *et al.,* 1997), presented a higher prevalence in adult horses (71,41%) than in the young horses (28,57%), but it is not probable that the prevalence can be linked to the host's age although these levels do not differ statistically according to Fischer's Exact Test.

Attention is called to the higher percentage rate of female specimens (Figure 2 and 3), often averaging above those of male specimens. It can be observed that the predominance of female specimens was even higher when estimated for the Cyathostominae nematodes found in the young hosts, in which they surpassed 78% of the total counts. This find corroborates previous assumptions that young hosts do carry a heavier female parasitic burden than the adult horses.

Concerning the forms still in development (immature forms), it was observed that previous assumptions were correct; adult hosts carried a lower cyathostomine burden ($P<0,01$) than the young ones. While adult hosts were infected with a little more than 2,000 of immature nematode forms, the young hosts carried a burden of above 50,000.

In closing, it can be said that our study adds to a vast bibliographic documentation of worldwide dimension on Cyathostominae in equids. Our research supports the fact that chemotherapeutic advances help suppress and control nematode infection in equid hosts. Despite the valuable contributions of geneticists to select host resistance against nematode infections, the horse and mule breeders of contemporary times are facing the conspicuously growing importance of the Cyathostominae. This group of parasites will need more and more veterinary research, since the small Cyathostominae are certainly the most troublesome parasites of Equidae.

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