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Ecological aspects of small strongylids in the Paraíba Valley Region, State of São Paulo, Brazil

Aspectos ecológicos dos pequenos estrongilídeos na região do Vale do Paraíba, Estado de São Paulo, Brasil

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ABSTRACT

Post-mortem *studies were conducted on twenty equids (16 horses and four mules) of the Paraíba Valley, during a period of twelve years (1988 to 2000) in order to establish the ecological descriptors of the different species of small strongylids (Subfamily Cyathostominae, Tribe Cyathostominea). Samples of 10% of total gastrointestinal content was examined and a total of 13,832 of Cyathostominea were obtained with a prevalence of 100%. The most prevalent and abundant species were* ***Cylicocyclus nassatus*** *(100%),* ***Cylicostephanus longibursatus*** *and* ***Cylicostephanus goldi*** *(95%). The population of Cyathotominea showed an average Dispersion Index (DI) of 49.03 and an average Green index (GI) of 0.226. The parasitic community had average diversity of 1.79, calculated by Shannon-Wiener' Index, and 0.77 by Simpson's Index of Diversity. The evenness (Pielou's Index) presented an average of 0.28.The intensity of infection had a positive correlation with the parasite richness (P<0,05) and the diversity (P<0,01). Specimens of* ***C. nassatus*** *showed preference for the ventral colon (P<0.01),* ***Cyathostomum coronatus*** *for the caecum (P<0.01),* ***C. longibursatus*** *and* ***C. goldi*** *a preference for both the ventral colon and the dorsal colon (P<0.01).*

Key words: *equids, Cyathostominae, ecology, São Paulo, Brasil.*

RESUMO

Foram realizados estudos post-mortem *em 20 eqüídeos provenientes do Vale do Paraíba para obtenção de descritores ecológicos dos pequenos estrongilídeos (Subfamíia, Cyathostominae, Tribo Cyathostominea). Das alíquotas de 10% do conteúdo intestinal total, foram recuperados 13.832 Cyathostominea com 100% de prevalência. As espécies,* ***Cylicocyclus. nassatus*** *(100%),* ***Cylicostephanus longibursatus*** *e* ***Cylicostephanus goldi*** *(95%) foram as mais prevalentes e abundantes. A população de Cyathostominea apresentou Índice de Dispersão (ID) médio de 49,03 e Índice de Green (IG) médio de 0,226. A diversidade apresentou média de 1,79 (Ìndice de Shannon - Wiener) e 0,77 (Índice de Diversidade de Simpson). O índice de Pielou registrou equitabilidade média de 0,28. A intensidade da infecção apresentou correlação positiva com a riqueza parasitária (P<0,05) e a diversidade (P<0,01). Espécies de* ***C. nassatus*** *mostraram preferência pelo cólon ventral (P<0,01),* ***Cyathostomum coronatus*** *pelo ceco (P<0,01),* ***C. longibursatus*** *e* ***C. goldi*** *preferência por ambos, cólon ventral e cólon dorsal (P<0,01).*

Palavras-chave: *eqüídeos, Cyathostominae, ecologia, São Paulo, Brasil.*

INTRODUCTION

Of all the gastrointestinal helminths that parasitise equids, the Cyathostominea, also known as small strongylids, present the biggest problem to their hosts. The Cyathostominea cause both clinical and subclinical disease. Clinical disease known as Cyathostomosis occurs when large numbers of encysted or hypobiotic larval re-emerge in the intestinal lumen. Symptoms of acute diarrhoea soon become chronic and weight loss, colic, subcutaneous oedema are seen (MAIR et al., 1994). This can be fatal in some cases (VAN LOON et al., 1995). The situation is worsened by the inefficiency of the anthelmintics like the avermectins which at the prescribed dose and even at 5 times the recommended dose do not have a

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significant effect against the encysted or hypobiotic larvae of these parasites (HERD & COLLES, 1995).

In Brazil, several investigative studies on prevalence and abundance regarding the Cyathostominae have already been published (LANFREDI, 1983; BARBOSA, 1995; OLIVEIRA et al., 1994; CARVALHO et al., 1998; SILVA et al., 1999; SOUTO MAIOR et al., 1999; RODRIGUES et al., 2000; ANJOS & RODRIGUES, 2006). However, only studies conducted in Rio de Janeiro (SOUTO MAIOR et al., 1999; RODRIGUES et al., 2000; ANJOS & RODRIGUES, 2006), considered the ecological analysis of the communities of equine parasites in the segments of the large intestine (caecum, ventral colon and dorsal colon) in individualised fashion.

Studies conducted in the Paraíba Valley in São Paulo have recorded a high prevalence (100%) of these parasites in equids in this region (PEREIRA & VIANNA, 2006). Thus, the main aim of the present study was to provide, as well as the usual data of prevalence and abundance, ecological descriptors of the Cyathostominea that parasitise equids in the Paraíba Valley Region. This data is of paramount importance for the adoption of effective measures for the control of these parasites, given that we have seen the appearance of new species within the Cyathostominea population that are resistant to anthelminthics (NILSSON et al., 1989; BORGSTEEDE et al., 1997).

MATERIAL AND METHODS

Between 1988 and 2000, twenty naturally infected equids, 16 horses and four mules, were subjected to necropsy and their gastrointestinal parasites identified. All animals in the study came from the Paraíba Valley Region. Comprehensive data about characteristics of the region, sanity, age brackets and regional origin of the animals can be found in PEREIRA & VIANNA (2006). On necropsy the animals had the large intestine removed and the various compartments (caecum, ventral and dorsal colon) were isolated and then opened, their contents were placed in a bucket. The organs were then washed and scraped into separate buckets. The floating content of the latter washings were then decanted. The sediment added to the bucket containing the total contents for each organ and 10% samples of each of these buckets were taken and homogenised, and then kept in formaldehyde (10%) at 60°C. Helminths were collected with the aid of a stereoscopic microscope, stored in a small jar containing formaldehyde and then identified through optic microscopy LICHTENFELS (1975), GEORGI (1982) and LICHTENFELS et al. (1998), temporarily mounted between slide and microslide after clearance in a 80% phenol solution in alcohol (LICHTENFELS, 1975).

The parasitic ecology concepts and terms used are those proposed by MARGOLIS et al. (1982) and BUSH et al. (1997). Data were obtained with regard to the male/female ratio, ecological descriptors, as Dispersion Index, the Green Index, the Shannon Index, and the Pielou Uniformity Index (LUDWIG & REYNOLDS, 1988) and Simpson's Index of Diversity (RODRIGUES, 2008).

The data regarding the worm count was transformed into Ln (x+1) and the correlations between the intensity of the infection and the Shannon' Index and Simpson' Index; intensity of the infection and parasite richness were analysed using the Pearson Correlation Test.

To establish the favourite site for each species, the intensities have been compared in each region of occurrence (caecum, ventral colon and dorsal colon), using analysis of variance and also using the Student *t* test. In these calculations, use has been made only of data arising from species that have been present in at least ten equids (COLOBERT-LAUGIER et al., 2002).

RESULTS

The total population of helminth collected in the digestive tract of equids and their prevalence and abundance were detailed in PEREIRA & VIANNA (2006). The present publication has limited its coverage to the species of tribe Cyathosthominea. These parasites were present in all the equids examined. A total of 13,832 helminths were found. Eight genera and 23 species were represented, with a prevalence of 100% and abundance of 691.60 helminths/animal examined (Table 1). In this study, the immature forms (larvae) have not been considered.

The most prevalent (100%) and abundant species (128.70 individuals/animal studied) was ***C. nassatus*** and the least prevalent (10%) ***Cylicocyclus elongatus*** (Table 1). The average proportion between the number of males and females collected was 1:2.01. Samples of ***C. nassatus*** showed the lowest proportion (1:0.65). The population of Cyathostominea showed a distribution standard of clustered distribution (Variance > Mean) with DI between 1.52 and 162.94, but with a low clustering index (a mean GI of 0.226), according to table 2.

The community had parasitic diversity media of 1.79, calculated by the Shannon- Wiener' Index, and

Table 1 - Quantitative descriptors of parasite populations of Cyathostominea collected from naturally infected equids (n=20) in the Paraíba Valley Region, State of São Paulo, Brazil (Sample of 10% of gastrointestinal contents).

Species	Prevalence (%)	Ratio specie/total helminths (%)	Intensity range	Relative abundance
Coronocyclus coronatus	90	5.99	1 – 192	41.45
C. labiatus	50	0.79	1 – 26	5.45
C. labratus	30	0.38	1 – 15	2.60
Cyathostomum catinatum	90	13.64	8 – 286	94.35
C. pateratum	80	6.22	1 – 286	43.00
Cylicocyclus ashworti	80	4.87	1 – 168	33.65
C. brevicapsulatus	40	0.10	1 – 3	0.70
C. elongatus	10	0.09	1 – 12	0.65
C. insigne	45	3.77	1 – 290	26.05
C. leptostomum	60	1.42	1 – 68	9.80
C. nassatus	100	18.61	4 – 384	128.70
C. radiatus	20	0.33	1 – 30	2.30
C. ultrajectinus	25	0.47	1 – 192	3.25
Cylicodontophorus bicoronatus	65	1.23	1 – 40	8.50
Parapoteriostomum euproctus	60	0.82	1 – 26	5.70
P. mettami	20	0.07	1 – 5	0.45
Petrovinema poculatum	35	0.12	1 – 4	0.80
Cylicostephanus minutus	90	2.67	1 – 76	18.45
C. calicatus	80	4.90	2 – 182	33.90
C. longibursatus	95	16.92	8 – 378	117.05
C. goldi	95	16.36	8 – 319	113.15
Poteriostomum ratzii	25	0.11	1 – 11	0.75
Poteriostomum imparidentatum	25	0.13	1 – 5	0.90
Total				691.60

0.77 by Simpson's Index of Diversity. The intensity of infection had a positive correlation with the parasite richness (r = 0.5460, P <0.05) and the diversity, both by the Shannon-Wiener' Index (r = 0.6421, P <0.01) as Simpson'Index (r = 0.7577, P <0.01). The evenness (Pielou' Index) presented similar between communities, ranging from 0.21 to 0.34 with an average of 0.28 (Table 3).

Most speciments recovered were on the ventral colon (57.4%), followed by the dorsal colon (29.6%) and caecum (15.7%). Out of all the compartments (Table 4), ***C. nassatus*** showed preference for the ventral colon (P<0.01) and ***C. coronatus*** for the caecum (P<0.01). In the cases of ***C. longibursatus*** and ***C. goldi*** there seemed to be a joint preference for the ventral and the dorsal colon (P<0.01), without any statistically significant difference in these compartments.

DISCUSSION

The above results confirm previously studies about the Cyathostominea and the wide prevalence and abundance of these parasites in equids. Out of the 52 currently recognised species of Cyathostominea that parasitise equids (LICHTENFELS et al., 2002), 23 were found in this study. Previous studies conducted in the region by VIANNA et al. (1988) and VIANNA & ARAÚJO (1989) identified 24 and 21 species of small strongylids parasites, in horses and mules. No reference is made to the prevalence and abundance of these parasites.

The high prevalence and abundance of the species ***C. nassatus*** found in this study is in agreement with the results found by other studies in Brazil (LANFREDI, 1983; OLIVEIRA et al., 1994; BARBOSA, 1995; CARVALHO et al., 1998; SILVA et al., 1999). Apart from ***C. nassatus***, species such as ***C. longibursatus*** and ***C. goldi*** and ***Cyathostomum catinatum***, ***Cylicostephanus minutus*** and ***C. coronatus*** have a higher prevalence and abundance in equids. The high incidence of these species is in line with the findings of other studies undertaken in this country (LANFREDI, 1983; OLIVEIRA et al., 1994; BARBOSA, 1995; CARVALHO et al., 1998; SILVA et al., 1999). The population of these species accounted for 75% of the Cyathostominea observed, confirming a feature of this group, where a large number of species coexist on one same host, with a predominance of several individuals of a few species (OUGBOURNE, 1976).

The low prevalence of ***C. elongatus*** in the animals of this region agrees with the findings of other

Table 2 - Aggregation indexes of Cyathostominea collected from naturally infected equids (n=20) in the Paraíba Valley region, State of São Paulo, Brazil (Sample of 10% of gastrointestinal contents). Dispersion Index (DI) and Green's Index (GI).

Species	DI	GI
Coronocyclus coronatus	61.45	0.07
C. labiatus	14.38	0.12
C. labratus	9.49	0.17
Cyathostomum catinatum	115.55	0.06
C. pateratum	133.23	0.15
Cylicocyclus ashworti	85.15	0.13
C. brevicapsulatus	1.52	0.04
C. elongatus	14.03	0.87
C. insigne	162.94	0.31
C. leptostomum	28.53	0.31
C. nassatus	92.56	0.14
C. radiatus	22.70	0.04
C. ultrajectinus	41.58	0.48
Cylicodontophorus bicoronatus	21.01	0.63
Parapoteriostomum euproctus	9.09	0.12
P. mettami	3.15	0.07
Petrovinema poculatum	2.32	0.27
Cylicostephanus minutus	34.37	0.09
C. calicatus	73.06	0.09
C. longibursatus	97.23	0.11
C. goldi	89.50	0.04
Poteriostomum ratzii	7.98	0.50
Poteriostomum imparidentatum	3.85	0.17
Mean	49.03	0.23
Standart Deviation	47.75	0.29

studies carried out in Brazil (LANFREDI, 1983, OLIVEIRA et al., 1994; SOUTO MAIOR et al., 1999), despite the species being described as common by LICHTENFELS (1975).

The average proportion observed between the numbers of males and females (1:2.01) was in agreement with the findings reported by BARBOSA (1995), CARVALHO et al. (1998), SILVA et al. (1999) and ANJOS & RODRIGUES, 2006. However, we must mention the low ration which this study found in the case of ***C. nassatus*** (1:0.65) or even in the works of some of the authors previously mentioned. As the species has always been reported as more prevalent and abundant, the lower number of females is probably not associated to this indicator. This relation can be seen for ***Petrovinema poculatum***, a species with low prevalence and abundance, for which we observed a low ratio (1:0.60), as, indeed, have other authors, including CARVALHO et al., (1998) (1:0.50), SILVA et al., 1999 (1:0.54) and BARBOSA, 1995, this latter study finding no females in 148 identified individuals.

The standard distribution clustering we have found, also found in other studies that have been concerned with the observation of this data, seems to be a rule for helmintths on equids (SOUTO MAIOR et al., 1999; RODRIGUES et al., 2000; ANJOS & RODRIGUES, 2006), which, according to SHAW & DOBSON (1995), is a standard feature of parasites. The Dispersion Index in terms of measuring the extent of clustering is not commonly used, as it is influenced by the number of individuals in the sample. The Green Index, apart from not being very dependent on the size of the sample, also estimates the dimensions of the clustering, on a scale from 0 (random distribution) to 1 (maximum clustering, should all the individuals be obtained from one same sample), according to JAYARAMAN (2008). Thus, in this case, the mean GI obtained, at 0.226, means a relatively low clustering standard for the population of Cyathostominea. The diversity is due to the richness of species (the number of species that live in a given habitat) and intensity of infection (size of populations). The Shannon-Wiener Index (H') gives greater weight to rarer species, while the Simpson Index (E) prefers the common species (BUSH et al. 1997). We obtained an average H' of 1.79, while the Simpson Index mean of 0.77, a value which suggests a high degree of diversity, as this index considers an interval between 0 and 1, as minimum and maximum diversity respectively. The Shannon' Index is adimensional.

The intensity of infection with Cyathostominea in equids shows positive correlation ($P< 0.05$) with the richness and the diversity of the species recovered ($P<0.01$), regardless of the index used for the calculation of the index (Shannon or Simpson), this being a fact which has also been confirmed by COLLOBERT-LAUGIER (2002) in France.

Equitability shows the way in which the number of individuals is distributed among different species. In our study, the mean was 0.28, suggesting low equitability, as the index considers the interval between 0 and 1, with 1 being the maximum value for uniformity (if all species have the same abundance).

There are no parameters in relevant literature about clustering, diversity and equitability specifically for Cyathostominea, within the total content of the large intestine in equids, and the observations that exist refer to individualised compartments.

Similar to our results and those of other publications, the dorsal colon and the ventral colon were preferred by most species (Le ROUX, 1924; FOSTER, 1936; FOSTER & ORTIZ, 1937; OGBOURNE, 1976; RODRIGUES et al., 2000; ANJOS & RODRIGUES, 2006). The preference of ***C. nassatus*** ($P< 0.01$) for the

Table 3 - Quantitative descriptors of parasites communities of Cyathostominea collected from naturally infected equids (n=20) in the Paraíba Valley Region, State of São Paulo, Brazil (Sample of 10% of gastrointestinal contents). Pearson's Correlation Coefficient (r).

Animals	Intensity of infeccion	Species richness	Simpson's Index (E)	Shannon's Index (H')	Pielou's Index (J')
01	1049	15	0.82	1.94	0.28
02	1083	18	0.81	2.00	0.29
03	1015	13	0.82	1.93	0.28
04	1121	17	0.81	1.82	0.26
05	982	8	0.74	1.47	0.21
06	1089	15	0.81	1.92	0.28
07	1061	14	0.85	2.02	0.29
08	869	14	0.78	1.77	0.26
09	72	6	0.44	0.89	0.21
10	1063	8	0.78	1.64	0.24
11	484	12	0.77	1.73	0.28
12	582	17	0.86	2.18	0.34
13	415	11	0.84	1.99	0.33
14	120	10	0.70	1.57	0.34
15	714	16	0.78	1.96	0.30
16	269	10	0.62	1.28	0.23
17	495	18	0.82	1.99	0.32
18	248	12	0.76	1.75	0.32
19	423	12	0.75	1.73	0.29
20	678	18	0.85	2.19	0.34
Mean	691.60	13.202	0.77	1.79	0.28
Standard Deviationn	358.88	3.6	0.10	0.31	0.04

Correlation between Intensity of infecction and Shannon' Index (r=0.6421, P<0.01).
Correlation between Intensity of infecction and Simpson'Iindex (r=0.7577, P<0.01).
Correlation between Intensity of infecction and Species richness (r=0.5460, P<0.05).

ventral colon is in conformity with other publications, like those of FOSTER (1936), FOSTER & ORTIZ (1937), OGBOURNE (1976), COLLOBERT-LAUGIER (2002) and ANJOS & RODRIGUES (2006). The double preference (P<0.01) shown by ***C. longibursatus*** and ***C. goldi*** for the ventral colon and the dorsal colon only disagrees with the findings of Le ROUX (1924), the only author to mention ***C. goldi*** as the species common in the caecum, a compartment where we have not found any examples of this parasite. The significant preference of ***C. coronatus*** for the caecum (P< 0.01) is a result which is unanimous among the authors who have been consulted. Even though most species of Cyathostominea show significant preference for a

Table 4 - Location in large intestine of Cyathostominea (species which were identified at least 10 horses - arithmetic mean) collected from naturally infected equids in the Paraíba Valley Region, State of São Paulo, Brazil (Sample of 10% of gastrointestinal contents). Student's *t*- test.

Species	Ventral colon	Dorsal colon	Caecum
Cylicocyclus nassatus	$163.00^{a*\,A**}$	$18.20^{b\,AB}$	$6.80^{b\,B}$
Cylicostephanus longibursatus	$54.67^{a\,A}$	$53.60^{a\,A}$	$0.27^{b\,B}$
Cylicostephanus goldi	$59.87^{a\,A}$	$48.60^{a\,A}$	$0.00^{b\,B}$
Cyathostomum catinatum	$55.73^{a\,A}$	$8.67^{b\,B}$	$13.40^{b\,B}$
Coronocyclus coronatus	$2.79^{b\,B}$	$0.80^{b\,B}$	$45.53^{a\,A}$
Cylicostephanus calicatus	$14.13^{a\,AB}$	$2.07^{b\,B}$	$23.33^{a\,A}$
Cylicocyclus ashworti	$31.00^{a\,A}$	$2.40^{b\,B}$	$3.13^{b\,B}$
Cyathostomum pateratum	$13.43^{a\,A}$	$5.14^{b\,AB}$	$2.00^{b\,A}$
Cylicocyclus leptostomum	$8.87^{a\,A}$	$0.67^{b\,A}$	$2.73^{ab\,A}$
Cylicostephanus minutus	$8.27^{a\,A}$	$1.07^{b\,B}$	$0.80^{b\,B}$

Means within lines with different letters are singnificantly different (*P<0.05, **P<0.01).

certain location within the intestine, the occurrence in the three compartments is commonly recorded, which can be explained by seasonality (OUGBOURNE, 1976) and the phase of development of the parasites (OUGBOURNE, 1976; FOSTER, 1936), which could occur anywhere in the intestine, with later migration of newly hatched adults to their favourite habitat (FOSTER, 1936). In spite of the fact that this is subject to speculation, biochemical and nutritional conditions are other factos which, according to OGBOURNE (1976), would explain the preference for certain locations in the intestine, as also the co-existence of several different species as parasites on the same host.

The population of Cyathostminea, from what we have been able to see, remains stable, with similar ecological indicators regardless of the time of year or the geographical location from where the samples were taken. The advent of powerful endectocides with wide spectrum of action, which entered the market in the 1980s, does not seem to have had much influence on the population of Cyathostominea, which, according to BOXELL (2004), happened with large strongylids.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

The authors state that the studies with animals were conducted in accordance with ethical standards.

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