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Research Note

Cyathostominae distribution in experimentally infected ponies

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Summary

Nine ponies were experimentally infected with third stage cyathostome larvae. These animals were examined post–mortem for small strongyle infections from October 2011 to January 2012. Seventeen species of the following Cyathostominae genera were identified: *Coronocyclus, Cyathostomum, Cylicocyclus, Cylicostephanus, Gyalocephalus* and *Petrovinema.* Of the 17 species of Cyathostominae recovered, *Cyathostomum catinatum, Cyathostomum alveatum* and *Cylicocyclus nassatus* were the most prevalent (100 %). From the entire population of Cyathostominae, 65.6 % were located in the caecum and only 34.4 % in the colon. Only 1,736 (24,6 %) females and 5,329 (75,4 %) males were observed. There was a significant difference between the number of males and females in the genus *Cyathostomum, Cylicocyclus* and *Gyalocephalus*.
Keywords: Cyathostominae; infection; pony; prevalence; distribution

Introduction

Nematodes of the subfamily Cyathostominae, known also as cyathostomes or cyathostomins, are common in Europe, and a large number of species have been observed (Collobert-Laugier *et al*., 2002; Hinney *et al*., 2011; Kuzmina *et al*., 2005; Kuzmina *et al*., 2007). These parasites are of enormous interest because of their major diversity, the high infection intensity and serious pathology associated with these infections. Cyathostominae consist of 52 different species, of which more than 40 have been described in horses (Lichtenfels *et al*., 2002). In individual animals, 8 – 12 common species usually account for the majority of the parasite burden (i.e.> 90 %) (Ogbourne, 1976).

Cyathostominae, like other gastrointestinal nematodes of the order Strongylida, are gonochorists with marked sexual dimorphism. Males of small strongyles are usually smaller and less numerous than females in cyathostomes of horses by Anjos and Rodrigues (2006); Mfitilodze and Hutchinson (1990) and Silva *et al*. (1999). In these studies, the female to male ratio was approximately 2:1. This sex ratio is also typical for strongyles in other hosts. For example, similar results for trichostrongylid nematodes of ruminants were published by Balicka-Ramisz *et al*. (2005); Bye and Halvorsen (1983); Garijo *et al*. (2009); Stien *et al*. (2005) and Suarez *et al*. (1995).

Cyathostomes have a direct nonmigratory life cycle. Third stage larvae ingested from pasture penetrate the wall of the large intestine and especially the colon, where development takes place before nematodes re-emerge and mature to adults within the lumen. Many researchers have described cyathostome preference for this part of the large intestine. Mfitilodze and Hutchinson (1985), Ogbourne (1976), Collobert-Laugier *et al*. (2002) and Lyons *et al*. (2011) reported that the highest number of species prefer the ventral colon to the dorsal colon and caecum. Ogbourne (1976) and Collobert-Laugier *et al*. (2002) reported the following preferred parts in descending order: dorsal colon (50 %; 64 %), ventral colon (45 %, 27 %) and caecum (5 %, 9 %). Only Stancampiano *et al*. (2010) published results which differed from previous studies: the ventral colon (67.34 %), the caecum (27.52 %) and the dorsal colon (5. 14 %).

The aim of this study was to assess the prevalence, abundance and distribution of Cyathostominae populations in the large intestine as well as the female to male ratio of the experimental infected ponies.

Material and Methods

Nine ponies (males), aged 18 months at the time of infection, were used in this study. The animals were housed in groups of three individuals in indoor boxes (30 m^2 per group). Common food (meadow hay, oats, NutriHorse Standard – Trouw Nutrition Biofactory Ltd., Prague – Horní Počernice) was provided. All animals were dewormed with a double dose (400 μg /kg IVM) of Noromectin 1.87 % Oral Paste for Horses and reared indoors in order to avoid natural cyathostome infection. Animals were monitored during the study using coprological methods (at 7 day intervals, later daily). From July 2011 to November 2011, cyathostome larvae were obtained from naturally infected horses for experimental infection. Freshly voided faeces were collected and incubated at room temperature for 10 days. Infective larvae were harvested by the Baermann technique, and following microscopic confirmation of the presence of cyathostome larvae, they were suspended in tap water and stored in a refrigerator at 4 °C until a sufficient number of larvae was acquired. The larvae were then stored in natural environmental conditions for 4 weeks to support their development. The infective larvae were put into clear plastic containers and placed out of direct sunlight, and the water was changed daily. Prior to administering the larvae to the ponies, viable larvae were pipetted to doses (30 000 larvae per animals) (Murphy & Love, 1997). The animals were euthanized using an intravenous administration of T-61 solution (Intervet, The Netherlands). The entire contents and mucosal tissue of the large intestine of each animal (colon, caecum) were examined and washed. These materials were kept in Barbagall solution (7.5 g NaCl; 30 ml formaldehyd; 1000 ml dd H2O) and later observed under a dissection microscope. Cyathostome specimens obtained from the entire contents of the large intestines were identified using an Olympus BX51 microscope. Nematodes from each part of the large intestine were illuminated, measured, and identified with the help of diagnostic keys, which were created based on previously published material of Anderson *et al*. (1980), Anjos and Rodrigues (2006); Baruš (1962); Lichtenfels *et al*. (1998; 2002; 2008). All of the experimental procedures were conducted in accordance with Czech legislation (section 29 of Act No 246/ 1992 Coll., on protection of animals against cruelty, as amended by Act No 77/2004 Coll.).

Table 1. Intensity, sex and distribution of cyathostome species in the large intestine

Species		Prevalence %	Intensity				Colon	Caecum	Total
			Min	Max	Mean	±SD	F/M	F/M	
Cyathostomum	*catinatum*	100	21	800	267.3	298.87	121/657	177/1451	**2406**
	pateratum	66.66	4	310	111.67	112.85	82/252	130/206	**670**
	alveatum	99.99	4	352	75.4	104.11	24/62	119/474	**679**
Cylicocyclus	*nassatus*	99.99	6	410	103.44	128.41	68/191	134/538	**931**
	insigne	66.66	1	70	28.83	25.48	10/34	25/104	**173**
	radiatus	55.55	1	26	8.8	8.01	25/12	5/2	**44**
	leptostomum	44.44	1	110	55.25	43.17	30/30	50/111	**221**
Coronocyclus	*coronatus*	77.77	1	110	39	40.75	104/88	16/65	**273**
	labratus	44.44	2	11	6.75	4.22	11/13	0/3	**27**
	labiatus	44.44	2	13	6.75	4.67	11/13	0/3	**27**
Cylicostephanus	*minutus*	66.66	1	256	88	101.38	7/35	241/248	**531**
	longibursatus	22.22	20	30	25	10.66	10/10	0/30	**50**
	calicatus	77.77	5	390	93.14	121.49	73/281	103/195	**652**
	asymericus	22.22	3	14	8.5	4.38	12/1	0/4	**17**
	hybridus	11.11	1	1	0.11	0.33	0/0	0/1	**1**
Gyalocephalus	*capitatus*	88.88	2	160	44.13	48.57	27/50	41/244	**362**
Petrovinema	*poculatum*	11.11	1	1	1	0.31	0/1	0/0	**1**

Results and Discussion

At present, Cyathostominae are the most common parasitic pathogens of horses (Dos Santos *et al*., 2011; Kuzmina, 2012; Slivinska *et al*., 2013). For this reason, many studies have been carried out on the infracommunity diversity of these gastrointestinal parasites. Cyathostominae currently comprise 13 genera and 52 species (Lichtenfels *et al*., 1998). In the majority of the European countries studied (Ukraine, Poland, Great Britain, Germany and Italy), the three most prevalent species were *Cylicocyclus nassatus, Cylicostephanus longibursatus* and *Cyathostomum catinatum* (Gawor, 1995; Hinney *et al*., 2011; Kuzmina *et al*., 2005; 2007; Ogbourne, 1976). Only Lichtenfels *et al*. (2001) found *Cylicocyclus ashworthi* to be the most abundant species in Scotland. *Cylicocyclus ashworthi* and *Cylicostephanus (Cylicotetrapedon) asymetricus* were recorded in high numbers in Ukraine (Kuzmina *et al*., 2005; 2007). Collobert-Laugier *et al*. (2002) reported *Poteriostomum imparidentatum* and *Cylicocyclus ultrajectinus* as the most prevalent species in France. In the Czech Republic (formerly Czechoslovakia), Prof. Baruš was the only scientist who studied combined cyathostomin infections. In the 1950's and 1960's, he identified the following most numerous and prevalent species (in decreasing order): *Cyathostomum catinatum, Cylicocyclus nassatus* and *Coronocyclus coronatus* (Baruš, 1958, 1962, 1963). The results of this study differ from those of Baruš (1958; 1962; 1963) due to the higher prevalence of *Gyalocephalus capitatus.* The species list in this study is not representative of all species in the Czech Republic because infection was obtained from a single source, however, it is interesting, that Baruš (1962) identified a similar number of cyathostome species. In this study seventeen species of the following Cyathostominae genera were identified: *Coronocyclus, Cyathostomum, Cylicocyclus, Cylicostephanus, Gyalocephalus* and *Petrovinema.* Of the 17 species of Cyathostominae recovered, *Cyathostomum catinatum, Cyathostomum alveatum* and *Cylicocyclus nassatus* were the most prevalent (100 %). The prevalence and mean intensity of each cyathostome species in the large intestine are shown in Table 1.

Only a few studies have been carried out on the site distribution of individual cyathostome species within the large intestine. Ogbourne (1976) and Collobert-Laugier *et al*. (2002) reported the most preferred parts as follows (in decreasing order): dorsal colon (Ogbourne, 1976 – 50 %; Collobert-Laugier *et al*., 2002 – 64 %), ventral colon (45 %; 27 %) and caecum (5 %; 9 %). Stancampiano *et al*. (2010), however, determined that the ventral colon (67.34 %), was the most preferred parts, followed by the caecum (27.52 %) and the dorsal colon (5.14 %). Reinemeyer *et al*. (1988) and Lyons *et al*. (2011) also observed the majority of adult worms in the ventral colon. Ogbourne (1976), Collobert-Laugier *et al*. (2002) and Mfitilodze and Hutchinson (1985) reported that a number of species showed a decreasing trend in the ventral colon (31; 11; 12), dorsal colon (30; 8; 5) and caecum (24; 2; 1). In this study, the majority of adult cyathostome nematodes (65.6 %) were concentrated in the caecum. The five most prevalent species in this part are given as follows in decreasing order: *Cyathostomum catinatum, Cyathostomum alveatum, Cylicocyclus nassatus, Cylicostephanus minutus* and *Cyathostomum pateratum.* Thirty-four percent of adult cyathostomes were found in the colon (dorsal colon and ventral colon). *Cyathostomum catinatum* was the most prevalent species in the colon followed by *Cylicostephanus calicatus, Cylicocyclus nassatus, Cyathostomum pateratum* and *Coronocyclus coronatus.* Seventeen cyathostome species were observed in both of these sections of the large intestine. Distribution of the adult cyathostome nematodes recovered from different parts of the large intestine is shown in Table 1.

In nematodes, female biased sex ratios among adult worms are commonly reported (Guyatt & Bundy, 1993; Poulin, 1997; Seidenberg *et al*. 1974). With respect to Cyahostominae, sex ratio is also often found to be female biased (Anjos & Rodrigues, 2006; Mfitilodze & Hutchinson, 1990; Silva *et al*., 1999). In Brazil, for example, Anjos and Rodrigues (2006) obtained 60.5 % females and 38.8 % males from the colon. Similarly, female nematodes in ruminants were dominant with respect to gastrointestinal nematodes of the order Strongylida (Balicka-Ramisz *et al*., 2005; Bye & Halvorsen, 1983; Garijo *et al*., 2009; Stien *et al*., 2005; Suarez *et al*., 1995). With regards to the sex ratio problem of the subfamily Cyathostominae, females were less numerous than males. Of the entire adult cyathostome population, 5,329 (75 %) were males and only 1,736 (25 %) were identified as females. The average number of males was greater than that of females in the majority of species, with the exception of *Cylicocyclus radiatus* and *Coronocyclus coronatus* in the caecum. A significant difference between the number of males and females was found in the genus *Cyathostomum, Cylicocyclus* and *Gyalocephalus* ($p < 0.01$). However, it is difficult to interpret this result. Paulin (1997) believes that the primary sex ratio in nematodes is usually unbiased, and mechanisms occurring later in life are likely to create a female bias in adults. One possible reason for the greater proportion of females is that they survive longer than males following infection (Paulin, 1997; Roche & Patrzek, 1966). Another factor for the interpretation of a male biased sex ratio in this study could be the greater vulnerability of "female" infective larvae. In natural conditions, horses are infected by both fresh and old infective cyathostome larvae that have been on pastures for long periods of time. In this study, horses received doses of infective cyathostome larvae that were exclusively made up of larvae of the same age. One explanation of this result may be that "female" larvae are more sensitive to storage and environmental conditions than are their male counterparts. Another explanation may be that male adult nematodes have shorter life spans in the intestine (Paulin, 1997; Roche & Patrzek, 1966), and it is possible there are higher numbers of males among infective larvae. However, both of these hypotheses have yet to be tested.

For obvious reasons, experimental gastrointestinal nematode infection in horses is very uncommon. Only three types of these studies have been published to date: Round (1969), who focused on the prepatent period of strongyles; Smith (1976), who dealt with reinfection of small strongyles; and Murphy and Love (1997), who examined the pathogenic effect of cyathostomes. However, none of these studies deal with sex ratio or localization. This study presents the results of an experimental cyathostome infection in nine ponies. As is evident, certain characteristics of reported experimental cyathostome infections differ from those of natural cyathostome infections. Further research into this type of experimental infection will be needed not only for horses but also for other herbivores.

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