SCREENING OF MODEL ANIMALS FOR EXPERIMENTAL INFECTION WITH EQUINE CYATHOSTOMES*

Š. Scháňková, I. Langrová, I. Jankovská, J. Vadlejch, Z. Čadková, D. Křivská

Czech University of Life SciencesPrague, Faculty of Agrobiology, Food and Natural Resources, Department of Zoology and Fisheries, Prague, Czech Republic

Various laboratory animals – mice (*Mus musculus*) of six strains, rabbits (*Oryctolagus cuniculus*), guinea pigs (*Cavia porcellus*), rats (*Rattus norvegicus*), and Mongolian gerbils (*Meriones unguiculatus*) were experimentally infected with larvae of small strongyles (Cyathostominae), obtained from horse faeces and cultured to the infective larval stage L3. The attempt to transfer cyathostome larvae was aimed at developing a model for the investigation of different aspects of the life cycle and biology of these nematodes in the laboratory. Some animals were immunized (hydrocortisone) for the duration of the study. The laboratory animals were orally infected with 2–10 thousand sheathed or ex-sheathed L3 larvae of mixed cyathostome species. All attempts to inoculate any animal failed; there was no larval development in the experimental rodents and it can be stated that none of the investigated animals may serve as a suitable model host for horse nematodes of the subfamily Cyathostominae.

small strongyles, cyathostomiasis, experimental models, rabbits, guinea pigs, Mongolian gerbils

DE GRUYTER OPEN

doi: 10.2478/sab-2018-0003
Received for publication on January 4, 2016
Accepted for publication on August 31, 2017

INTRODUCTION

On a worldwide basis, horses are exposed to a complex of intestinal helminth infections which can compromize their health and welfare. For instance, there is a high prevalence of nematodes of the subfamily Cyathostominae (Strongylida), which represents the most common group of horse parasites being a significant cause of morbidity and mortality. These intestinal helminths are difficult to control, not least due to the lack of information available regarding the basic biology. The cyathostomes, also known as small strongyles, include 14 genera with over 50 species, of which more than 40 have been described in horses (Lichtenfels et al., 2008). They have a direct, *non-migratory* life cycle without intermediate hosts (Corning, 2009). In the individual host, 8–12 common species usually account for the majority of the parasite burden (i.e. > 90%). Virtually, every horse can most likely become infected with these parasites, which often accounts for a parasitic load of up to 100% in grazing pastures (Love et al., 1999; Kaplan, 2002; Lyons et al., 2011). The infective stage able to survive a prolonged period in the pasture is represented by the third stage larvae (L3), surrounded by a protective membrane. After ingestion, the L3s exsheath in the small intestine and penetrate the wall of the large intestine and especially the colon. There the development takes place, before nematodes re-emerge and mature to adults within the lumen. Cyathostominae

* Supported by the Ministry of Agricultute of the Czech Republic, Project No. QJ1510038, and by the Internal Grant Agency of the Czech University of Life Sciences Prague (CIGA), Project No. 20142030.

Table 1. Results of cyathostome development in experimental models

Species of experimental models		n	Infected dose [*n*]	Investigated group of experimental models		
				L3[a]	L3[a]/hydrocortisone	ex-sheathed L3[a]/hydrocortisone
Mus musculus	C57 bl-10	8	2 000	negative	negative	negative
	Balb C3-MAN	8	2 000	negative	negative	negative
	DBA 2	8	2 000	negative	negative	negative
	C3H/DisN	8	2 000	negative	negative	negative
	C57/Bl 60	8	2 000	negative	negative	negative
	A/Ph	8	2 000	negative	negative	negative
Oryctolagus cuniculus		8	10 000	negative	negative	negative
Cavia porcellus		6	10 000	negative	negative	negative
Rattus norvegicus		8	2 000	negative	negative	negative
Meriones unguiculatus		6	2 000	negative	negative	negative

[a] third larval stage

can be non pathogenic as well as extremely pathogenic, and high levels of infection result in clinical symptoms ranging from chronic weight loss to colic, diarrhoea or severe inflammatory enteropathy (Murphy, Love, 1997; Lichtenfels et al., 2002; Matthews et al., 2004; Stratford et al., 2011).

Experimental work with horses is extremely difficult for many reasons. Finding suitable model hosts for a group of equine small strongyles, as with trichostrongylids of ruminants, can shed significant light on biology of this parasite group of veterinary importance (Ziam et al., 1999; Jankovska et al., 2003a, b). Therefore, the main objective of this study was to investigate the susceptibility of five laboratory animals to cyathostome parasites.

MATERIAL AND METHODS

In this study, five small species of mammals – mice (*Mus musculus*), rabbits (*Oryctolagus cuniculus*), guinea pigs (*Cavia porcellus*), rats (*Rattus norvegicus),* and Mongolian gerbils (*Meriones unguiculatus*) were used as experimental models; six strains of white mouse were involved. All the animals were kept in a biosafety breeding room under controlled temperature and humidity, fed commercial pellets, and supplied with water *ad libitum*. Some animals were switched from commercial diet to a modification of commercial diet containing 0.02% hydrocortisone (medicated) for the duration of each study.

Cyathostome larvae were obtained from naturally infected horse. Freshly voided faeces were collected and incubated at room temperature for 10–12 days. Infective larvae were harvested using the Baermann technique, the presence of cyathostome larvae was microscopically confirmed. The larvae were suspended in tap water and stored in a refrigerator at 4°C until a sufficient number of larvae were acquired (maximum 2 weeks). Before using for animals infecting, L3 larvae were divided into the following groups: sheathed larvae left without treatment and larvae ex-sheathed using sodium hypochlorite (Zinsli, 1987). The overall experiment pattern is shown in Table 1.

RESULTS

None of the individuals of the five laboratory species (mice, rabbits, guinea pigs, rats, and Mongolian gerbils) used as experimental models was successfully infected with nematodes of the subfamily Cyathostominae. All attempts to inoculate these mammal species with variously treated larvae failed, regardless of the experimental group (Table 1).

No rodent was successfully infected with nematodes.

DISCUSSION

Testing the cyathostome larvae transfer to laboratory rodent models was inspired by reports on a successful infection of various laboratory animals with relatively related trichostrongylid nematodes of domestic ruminants (Strongylida: Trichostrongylidae). For example, Conder et al. (1991, 1992) studied the susceptibility of gerbils to Haemonchus contortus, which is commonly found in sheep, goats, and cattle. Gerbils, rabbits, and guinea pigs have also been successfully used as experimental models in many studies focused on susceptibility to Trichostrongylus colubriformis (Court, Lees, 1985; Bezubik et al., 1988; Rothwell et al., 1994; Ziam, Pandey, 2000; Audebert et al., 2003; Jankovska et al.,

2003a, b). In all cases, the highest activity was detected immediately after inoculation with ex-sheathed infective larvae, and also during the hydrocortisone dosing phase.

The evolutionary mechanisms vary among parasite groups. While trichostrondylids (Trichostrongylidae) used dispersion within different hosts or host change, horse cyathostomes (Strongylidae) utilized coevolution (Durette-Desset et al., 1994). The strict host specificity of Cyathostominae may probably be attributed to a long common evolution with their hosts. Therefore, the experimental infection has never been achieved with the only one cyathostome species and only mixed cyathostome species thus have to be used for this purpose (Klei, Chapman, 1999).

CONCLUSION

In this study, five laboratory mammals (mice, rats, rabbits, guinea pigs, and gerbils) were utilized as experimental models in order to verify their susceptibility to nematodes of the subfamily Cyathostominae. All attempts to inoculate these laboratory species failed. We may conclude that none of the investigated species can serve as a suitable model host for nematodes of the subfamily Cyathostominae.

ACKNOWLEDGEMENT

The authors are very grateful to Brian Kavalir for his valuable help in language correction.

REFERENCES

Audebert F, Cassone J, Kerboeuf D, Durette-Desset MC (2003): Development of Trichostrongylus colubriformis and Trichostrongylus vitrinus, parasites of ruminants in the rabbit and comparison with Trichostrongylus retortaeformis. Parasitology Research, 90, 57-63. doi: 10.1007/s00436-002-0820-3.

Bezubik B, Wedrychowicz H, Wojciechowska A (1988): Trichostrongylus colubriformis in rabbits: some quantitative aspects and pathogenesis of single and multiple infections. Acta Parasitologica Polonica, 33, 131-142.

Conder GA, Johnson SS, Guimond PM, Geary TG, Lee BL, Winterrowd CA, Lee BH, DiRoma PJ (1991): Utility of a Haemonchus contortus/jird (Meriones unguiculatus) model for studying resistance to levamisole. Journal of Parasitology, 77, 83–86. doi: 10.2307/3282562.

Conder GA, Johnson SS, Hall AD, Fleming MW, Mills MD, Guimond PM (1992): Growth and development of Haemonchus contortus in jirds, Meriones unguiculatus. Journal of Parasitology, 78, 492–497. doi: 10.2307/3283650.

Corning S (2009): Equine cyathostomins: a review of biology, clinical significance and therapy. Parasites and Vectors, 2 (Suppl. 2), Article S1. doi: 10.1186/1756-3305-2-S2-S1.

Court JP, Lees GM (1985): The efficacy of benzimidazole anthelmintics against late fourth stage larvae of Trichostrongylus colubriformis in gerbils and Nippostrongylus brasiliensis in rats. Veterinary Parasitology, 18, 359–365. doi: 10.1016/0304-4017(85)90070-6.

Durette-Desset MC, Beveridge I, Spratt DM (1994): The origins and evolutionary expansion of the Strongylida (Nematoda). International Journal of Parasitology, 24, 1139–1165. doi: 10.1016/0020-7519(94)90188-0.

Jankovska I, Langrova I, Vadlejch J (2003a): Arrested development of Trychostrongylus culubriformis in experimentally infected rabbits. The effect of acquired resistance, single, multiple doses and size of doses on rise of hypobiosis. Scientia Agriculturae Bohemica, 34, 58–62.

Jankovska I, Langrova I, Fucikova A, Borovsky M (2003b): Effect of parasitic nematodes Trichostrongylus colubriformis on haematological parameters of model host (Oryctolagus cuniculus f. domestica). Scientia Agriculturae Bohemica, 34, 94–98.

Kaplan RM (2002): Anthelmintic resistance in nematodes of horses. Veterinary Research, 33, 491–507. doi: 10.1051/vetres:2002035.

Klei TR, Chapman MR (1999): Immunity in equine cyathostome infections. Veterinary Parasitology, 85, 123–136. doi: 10.1016/S0304-4017(99)00093-X.

Lichtenfels JR, Gibbons LM, Krecek RC (2002): Recommended terminology and advances in the systematics of the Cyathostominea (Nematoda: Strongyloidea) of horses. Veterinary Parasitology, 107, 337–342. doi: 10.1016/S0304-4017(02)00167-X.

Lichtenfels JR, Kharchenko VA, Dvojnos GM (2008): Illustrated identification keys to strongylid parasites (Strongylidae: Nematoda) of horses, zebras and asses (Equidae). Veterinary Parasitology, 156, 4–161. doi: 10.1016/j.vetpar.2008.04.026.

Love S, Murphy D, Mellor D (1999): Pathogenicity of cyathostome infection. Veterinary Parasitology, 85, 113–122. doi: 10.1016/S0304-4017(99)00092-8.

Lyons ET, Kuzmina TA, Tolliver SC, Collins SS (2011): Observations on development of natural infection and species composition of small strongyles in young equids in Kentucky. Parasitology Research, 109, 1529–1535. doi: 10.1007/s00436-011-2460-y.

Matthews JB, Dowdall SM, Baudena MA, Klei TR, Kaplan RM, von Samson-Himmelstjerna G, Drogemuller M, Schnieder T (2004): Equine cyathostomins. Veterinary Parasitology, 125, 203–220.

Murphy D, Love S (1997): The pathogenic effects of experimental cyathostome infections in ponies. Veterinary Parasitology, 70, 99–110. doi: 10.1016/S0304-4017(96)01153-3.

Rothwell TLW, Wagland BM, Sangster NC (1994): Expulsion of Trichostrongylus colubriformis by high and low responder guinea-pigs. International Journal for Parasitology, 24, 527–531. doi: 10.1016/0020-7519(94)90144-9.

Stratford CH, McGorum BC, Pickles KJ, Matthews JB (2011): An update on cyathostomins: anthelmintic resistance and diagnostic tools. Equine Veterinary, 39 (Suppl. 39), 133–139. doi: 10.1111/j.2042-3306.2011.00397.x.

Ziam H, Pandey VS (2000): Duration of infection of jirds (Meriones unguiculatus) with Trichostrongylus colubriformis. Journal of Parasitology, 86, 893–896. doi: 10.2307/3284993.

Ziam H, Pandey VS, Darwiche J, Losson B, Kumar V (1999): Biological parameters of Trichostrongylus colubriformis in Meriones unguiculatus. Veterinary Parasitology, 81, 309–322. doi: 10.1016/S0304-4017(98)00255-6.

Zinsli M (1987): In vitro cultivation of Ostertagia leptospicularis (Trichostrongylidae) with studies on hypobiosis. Dissertation, University of Zürich. (in German)

Corresponding Author:

prof. Ing. Iva L a n g r o v á , CSc., Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Department of Zoology and Fisheries, Kamýcká 129, 165 00 Prague 6-Suchdol, Czech Republic, phone: +420 224 382 673, e-mail: langrova@af.czu.cz