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Baylisascaris transfuga (Ascaridoidea, Nematoda) from European brown bear (*Ursus arctos*) causing larva migrans in laboratory mice with clinical manifestation

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Abstract

Due to the recent recovery of brown bear populations in Central Europe, information about their ascarid parasite, *Baylisascaris transfuga* is necessary as the parasite represents a part of natural ecological networks. *B. transfuga* can lead to larva migrans syndrome in accidental hosts, but its zoonotic potential has not been confirmed. The resent study compares development of larva migrans in infected mice inoculated with two infectious doses (ID 200 and ID 2000) of *B. transfuga* embryonated eggs, and the clinical manifestation to evaluate the pathogenicity of the larvae. Histopathology revealed that the liver was the most severely infected organ. The moderately infected organs included lung, brain, skeletal muscles and jejunum and the less infected ones were the eyes, heart, kidneys and spleen. The high pathogenicity of *B. transfuga* to mice was reflected in high mortality (33,3%) after infection, with mortality increasing with higher infectious dose. The results extend the knowledge of the interaction of *B. transfuga* and its aberrant hosts and contribute to the understanding of the epidemiology and transmission of this bears roundworm.

Keywords *Baylisascaris transfuga* · Brown bear · Larva migrans · Mice

Introduction

Parasitic nematodes of the order Ascaridida are considered important for veterinary and human medicine worldwide, not only because of the long-term viability of their highly resistant eggs but also because of the somatic migration of their larvae in a wide host range. *Ascaris*, *Baylisascaris* and *Toxocara* species are considered the most common cause of clinical larva migrans in animals and humans, which can be associated with severe or fatal disease. *Baylisascaris transfuga* (Rudolphi, 1819) represents the most common ascarid species in Carpathian brown bears (*Ursus arctos arctos*, Linné, 1758) in Slovakia (Štrkolcová et al. 2018). Recently, the brown bear population in Central Europe has been recovering thanks to species conservation, and thus, *B. transfuga* can spread along with thriving of its host. *B. transfuga* appears to have primarily a direct life cycle, but can lead to larva migrans syndrome in accidental hosts. The risk of clinical disease from *B. transfuga* larva migrans in aberrant hosts is considered low (Sapp et al. 2017). To date, there are two recent reports from natural conditions in wild rodents (Bugmyrin and Spiridonov 2019) and moose (Hoberg et al. 2018), with further sporadic information from experiments

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in laboratory animals. *B. transfuga* larvae have been shown to be capable of migrating in mice, guinea pigs, Mongolian gerbils and chickens (Matoff and Komandarev 1965; Papini et al. 1993, 1996; Sato et al. 2004). In these studies, high doses of *B. transfuga* embryonated eggs (1000–5000 or up to 30,000) were used for experimental inoculations with different observations of the clinical condition and behaviour of the larvae in different hosts. Compared to related *Baylisascaris* species, especially the raccoon roundworm *B. procyonis* (Stefanski and Zarnowski, 1951), larva migrans of *B. transfuga* is generally regarded less pathogenic (Papini and Casarosa 1994; Sato et al. 2004), and no human case of larva migrans caused by this species has been reported so far (Bauer 2013).

The aim of the present study is to compare the outcomes of two infectious doses of *B. transfuga* eggs in laboratory mice and the development of larva migrans including clinical manifestation, and to evaluate the pathogenicity of the lower infectious dose for mice aberrant hosts.

Material and methods

Experimental inoculation

Adult *B. transfuga* nematodes were obtained from the small intestine of an adult male roadkill brown bear from Malá Fatra National Park (Lipovec locality), Slovakia and provided by the State Nature Conservancy of the Slovak Republic. Eggs were obtained from adult females by dissection of their uteri and incubated at laboratory temperature (21 °C) for 30 days to obtain embryonated eggs, which were microscopically examined to assess their viability and counted.

Thirty-five subadult laboratory mice (females, CD1 strain) were housed in animal facility at the Department of Pathological Morphology and Parasitology, University of Veterinary Sciences Brno in accordance with Czech legislation (Act No 246/1992 Coll., on the Protection of Animals from Cruelty). The mice were kept on commercial pelleted food and water ad libitum. All experimental procedures were performed in accordance with Protocol 10–2015 approved by UVS and Central Commission for Animal Welfare, Czech Republic. Mice were divided into 3 experimental groups. The first group included 15 mice; each mouse was inoculated with a dose of 200 embryonated eggs (ID 200). The second group consisted of 15 mice, each inoculated with 2 000 embryonated eggs (ID 2000). All mice were inoculated orally by gavage with purified and counted viable eggs of *B. transfuga* suspended in drinking water (0.2 ml/mouse). The third group of 5 mice served as uninfected negative control. All mice were checked daily for 8 weeks after inoculation by observation in their home cage (activity, observable abnormalities of general health and well-being) for the presence of clinical sings of the disease. Dead or euthanized mice were dissected and their organs [eyes, brain, heart, lungs, liver, jejunum, kidneys, spleen and skeletal muscles (triceps, quadriceps)] were processed for histopathological examination: fixed in 10% buffered formalin, embedded in paraffin, sectioned and stained with haematoxylin and eosin.

Molecular analyses

Total DNA was isolated from adult *B. transfuga* females using a NucleoSpin Tissue kit (Macherey Nagel, Düren, Germany) following the manufacturer's instructions. The DNA markers used for identification were as follows: internal transcribed spacers 1, 5.8S and internal transcribed spacer 2 (ITSs); Cytochrome c oxidase subunit I (COI) and 28S ribosomal RNA gene (28S). The primers used are shown in Table 1. All polymerase chain reactions (PCR) were prepared in a final volume of 25 µl containing 12.5 µl of PCR-BIO Taq Mix Red (PCR Biosystems), 20 pmol of forward and reverse primer, 8.5 µl of PCR water and 2 µl of the DNA (10–20 ng). Cycling conditions are described in Table 2. PCR products were visualized on a 2% agarose gel stained with Midori Green Advance (Elisabeth Pharmacon, Czech Republic). PCR amplicons were purified with the Gel/PCR DNA Fragments Extraction Kit (Geneaid Biotech, Taiwan) and sequenced commercially by Macrogen (Amsterdam, The Netherlands) using the indicated PCR primers.

ITSs that yielded sequences with mixed chromatograms were subsequently cloned using pGEM®-T Easy Vector Systems (Promega Corporation, USA). The cloned plasmid DNA was purified from bacterial culture using the GenElute™ Plasmid Miniprep Kit (Sigma-Aldrich, USA) and sequenced using universal T7/SP6 primers. Sequences were

Table 1 PCR primers used in this study

Marker	Primers	Sequence	Reference
28S	28S rDNA F	5′-CGAGGATTCCCTTAGTAACT-3′	Franssen et al. (2013)
	28S rDNA R	5′-TCGGATAGGTGGTCAACG-3′	
COI	JB3	5′-TTTTTTGGGCATCCTGAGGTTTAT3′	Bowles et al. (1992)
	JB 4.5	5′-TAAAGAAAGAACATAATGAAAATG-3′	
ITSs	18sF	5′-ACTGCTGTTTCGAGACCTTTCGAG-3′	Testini et al. (2011)
	28sR	5′-TAGCACCTTCTTTGGACTATAGCC-3′	

Table 2 PCR cycling conditions used for different markers of DNA *Baylisascaris transfuga*

Marker	Preheating	PCR 35 cycles of			Final extension
		Denaturation	Annealing	Extension	
28S	95 °C, 60 s	95 °C, 15 s	55 °C, 30 s	72 °C, 60 s	72 °C, 5 min
COI			55 °C, 30 s	72 °C, 30 s	
ITSs			61 °C, 30 s	72 °C, 30 s	

edited and assembled using the Geneious Prime software. Contigs were compared with those in the GenBank database by BLASTn analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Differences in parasite invasion in particular tissues were analysed using the chi-square test.

Results

In the group of mice infected with ID 200, three individuals died between DPI (days post inoculation) 2–10, and twelve mice were euthanized between DPI 7–56. The second group of mice infected with ID 2000 included seven mice that were found dead between DPI 6 and 7; the remaining eight mice were euthanized between DPI 7–9 due to severe clinical symptoms of infection (Table 3). Overall, 10 out of 30 (33,3%) of the infected mice died after experimental infection. All five mice in the negative control group showed no clinical symptoms and were euthanized sequentially: the first mouse at DPI 7, the second mouse at DPI 14 and remaining three mice at DPI 56.

Six mice (ID 200) were asymptomatic, four showed mild clinical symptoms such as roughened fur and moderate symptoms such as laboured breathing and five mice developed severe symptoms such as apathy, torticollis, immobility and ascites along with laboured breathing and roughened fur. Three mice also showed slowing of movements on the left side only. In the group of ID 2000 mice, seven mice died peracutely without clinical symptoms being observed and eight mice had to be euthanized due to apathy, atypical posture and respiratory problems (accelerated laboured abdominal breathing). The last euthanized mouse with ID 2000 developed severe clinical symptoms such as apathy, inability to move, ascites, also with laboured breathing and roughened fur.

Histological examination of ID 200 mice revealed larval stages in only some of the organs examined (liver, brain, skeletal muscles, lung, jejunum and spleen; Table 3). However, in the group of ID 2000 mice, larvae were found larvae in all organs examined except the spleen (Table 3). Overall, the most invaded organ was the liver, where the presence of larvae was statistically significant compared to other organs examined ($p < 0.05$). Moderately infected organs were lung, brain, skeletal muscles and jejunum and least frequently larvae were detected in the eyes, heart, kidneys and spleen ($p < 0.05$).

In general, acute, subacute and subchronic histopathological changes were observed in mice from DPI 7. Between DPI 10 and 56 subchronic and chronic changes were detected, 56 DPI only chronic inflammatory changes and fibrosis were noted.

Pathological changes in the liver of mice examined up to DPI 9 included acute focal changes with inflammatory infiltrates (neutrophils), multifocal chronic active inflammation with abscesses and focal necrosis with a mixed inflammatory infiltrate of macrophages, lymphocytes, plasmatic cells and neutrophils. Larvae were detected in the liver parenchyma and vessels. Mice examined between DPI 10 and 56 showed predominantly chronic active inflammation with abscesses and necrosis, moreover focal or diffuse vacuolar and hydrophic dystrophy of hepatocytes with larvae in the liver parenchyma. Histopathological changes in the liver were observed in 10 of 15 ID 200 mice and in 13 of ID 2000 mice.

Histopathological changes were found in the lungs of 11 ID 200 mice and 13 ID 2000 mice. Findings included haemorrhage, multifocal chronic purulent pneumonia, fibroplasia, inflammatory infiltrates with macrophages and eosinophils. Histopathological changes of various intensity were detected from DPI 7; subchronic and chronic inflammatory changes were observed between 10 and 56 DPI.

Forelimb and hindlimb skeletal muscle exhibited focal chronic active inflammation and fibroplasia with inflammatory infiltrates containing neutrophils, lymphocytes, macrophages and central larvae in mice examined by DPI 9. In mice examined at the 10^{th} and 14^{th} DPI, histopathology showed abscesses in skeletal muscles and chronic inflammation with fibroplasia and larvae in fibrotic tissue at the 56^{th} DPI. Inflammatory changes were detected in 4 (triceps) and 7 (quadriceps) ID 200 mice and in 2 (triceps) and 3 (quadriceps) ID 2000 mice.

Chronic inflammatory infiltrates were present in the kidneys in DPI 7–10 in 2 ID 200 mice and 4 ID 2000 mice.

Despite the presence of larvae, brain tissue was without pathological changes in most mice; abscesses were detected only in two symptomatic mice (euthanized 10 DPI and 56 DPI), and in two asymptomatic mice (56 DPI), a focal-mixed inflammatory infiltrate with neutrophils, eosinophils, lymphocytes and plasmatic cells was observed.

Table 3 Mice experimentally inoculated with *Baylisascaris transfuga* eggs. *D* found dead, *E* euthanized, *DPI* days post infection, *ID* infectious dose (number of embryonated eggs used for experimental inoculation), *S* symptoms (*x* = found dead with no symptoms previously observed, + mild symptoms, + + moderate symptoms, + + + severe symptoms, *no* no symptoms observed at all), *PF* pathological findings (*A* autolysis, *N* no findings during necropsy, *Y* macroscopic pathological findings at necropsy), *L* larva found, dark grey colour inflammatory changes. Mice 19, 28, 33–35 served as negative controls with no symptoms/pathological findings

Mouse no	D/E	DPI	ID	S	PF	Left eye	Right eye	Brain	Heart	Lung	Liver	Spleen	Jejunum	Kidney	Triceps	Quadriceps
1	D	2	200	no	N											
16	E	7	200	+	Y			L		L	L					
17	E	7	200	+	Y			L			L					
18	E	7	200	+	Y			L		L	L					
21	E	9	200	+ + +	Y						L				L	
22	E	9	200	+ + +	Y										L	L
23	E	10	200	+ + +	Y						L					
24	E	10	200	+ + +	Y						L					
25	E	10	200	+ + +	Y						L	L				
26	D	10	200	no	A					L						
27	D	10	200	no	A					L	L		L			L
29	E	14	200	+	Y			L			L					
30	E	56	200	no	N			L								
31	E	56	200	no	Y											
32	E	56	200	no	Y			L							L	L
2	D	6	2000	x	Y	L	L	L		L			L			L
3	D	6	2000	x	A			L	L		L					
4	D	6	2000	x	A	L			L	L			L			
5	D	6	2000	x	Y					L	L				L	
6	D	6	2000	x	Y			L			L		L			
7	E	7	2000	+ + +	Y				L		L				L	
8	E	7	2000	+ + +	Y						L		L	L	L	
9	E	7	2000	+ + +	Y			L		L	L			L	L	
10	E	7	2000	+ + +	Y				L	L	L					
11	E	7	2000	+ + +	Y			LL		L			L			
12	E	7	2000	+ + +	Y	L		L	L		L		L			
13	E	7	2000	+ + +	Y			L		L	L		L			
14	D	7	2000	x	A					L	L		L			
15	D	7	2000	x	A					L	L		L			
20	E	9	2000	+ + +	Y						L					
19	E	7	0	no	N											
28	E	14	0	no	N											
33	E	56	0	no	N											
34	E	56	0	no	N											
35	E	56	0	no	N											

The heart was without pathological changes in most mice; only two ID 200 mice 10 DPI and 56 DPI showed a focal chronic active infiltrate in the myocardium.

In the spleen, reactive hyperplasia of lymphoid follicles was observed in mice between DPI 10 and 56 in 11 ID 200 mice and two ID 2000 mice. The jejunum and eyes were without pathological changes in all examined mice. Table 3 shows that inflammatory changes occurred in the lung, kidney, liver, brain, skeletal and cardiac muscle tissues were present in some mice without findings of larvae, indicating a tissue reaction to the previous presence or migration of larvae. Histological sections of *B. transfuga* larvae in the mouse brain (Fig. 1a), heart (Fig. 1b), liver (Fig. 1c) and lung (Fig. 1d) are depicted.

The final sequences of 28S, COI and ITSs were obtained and deposited in the GenBank database under accession numbers MW026407–MW026411. All sequences obtained showed identity with the sequences of *B. transfuga* available in the GenBank database (Table 4).

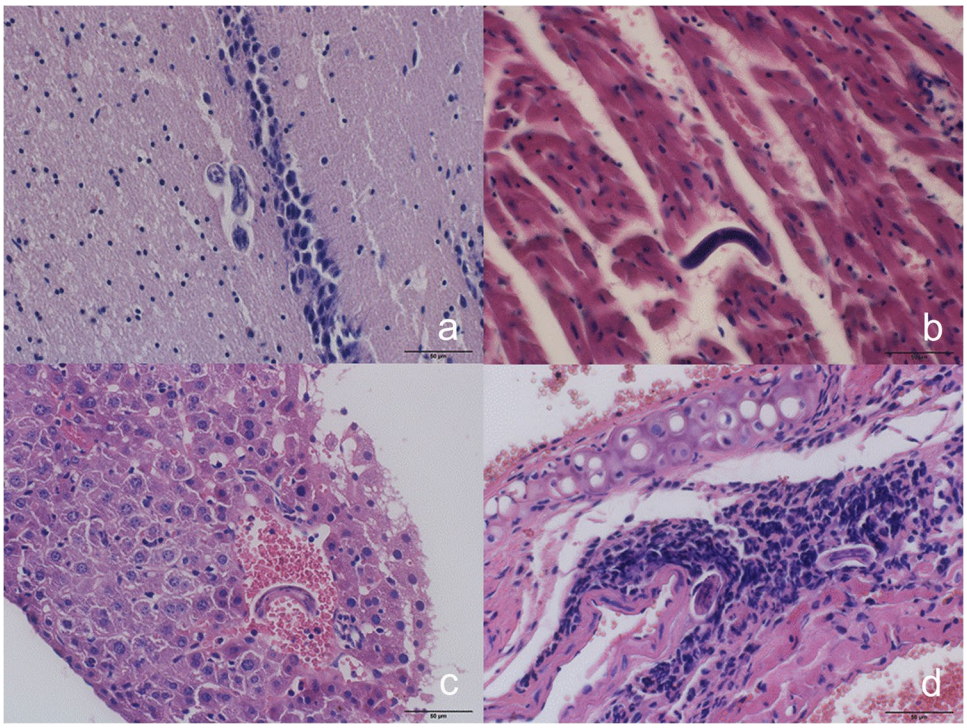

Fig. 1 Histological sections of *Baylisascaris transfuga* larvae in tissues from experimentally infected mice, HE stain, scale = 50 µm. **a** Brain tissue with larval stages without local pathological changes (mouse no 3, ID 2000, 6 DPI), **b** myocardium with larva located between cardiomyocytes, without pathological changes of the host tissue (mouse no 3, ID 2000, 6 DPI), **c** liver parenchyma with larva in dilated hepatic capillary lumen (mouse no 7, ID 2000, 7 DPI), **d** lung with larvae in peribronchial connective tissue with mixed inflammatory infiltrate (mouse No. BT5, ID 2000, 6 DPI)

Table 4 Comparison of the *Baylisascaris transfuga* sequences from the GenBank database showing identity with the sequences of the isolate used in this study, deposited in the GenBank database under accession numbers MW026407–MW026411

Marker	Base pairs	Sequence acc. No	% of identity to sequence (acc. No)	Host species	Collection location
28S	713	MW026407	100 (KC543471)	*Ursus arctos*	Netherlands
			100 (JN257009)	*U. arctos pruinosus*	China
COI	412	MW026408	99,76 (KC543477)	*Melursus ursinus*	Netherlands
			99,51 (HQ671079)	*U. maritimus*	China
ITS 1	1212	MW026409	99,34 (MH030602)	*U. americanus*	USA
			99,21 (MK496546)	*Microtus oeconomus*	Russia
ITS 2	1211	MW026410	99,32 (MK496546)	*M. oeconomus*	Russia
			99 (MH030602)	*U. americanus*	USA
ITS 3	1210	MW026411	99,34 (MH030602)	*U. americanus*	USA
			99,32 (MK496546)	*M. oeconomus*	Russia

Discussion

The present results indicate a high pathogenicity of *B. transfuga* from the European brown bear for experimentally infected mice. To simulate possible natural conditions under which *Baylisascaris* eggs may be spread in the environment, a lower dose (200 embryonated eggs) was used to be compared with the higher dose (2000 eggs) similar to those used in previous experimental studies (Matoff and Komandarev 1965; Papini et al. 1996; Sato et al. 2004).

In mice, Matoff and Komandarev (1965) reported that larvae of *B. transfuga* migrate by three routes: (1) in the intestinal wall, where they encyst; (2) through the intestinal wall into the peritoneal cavity and into the mesenteries, abdominal and thoracic organs; and (3) from the intestinal wall into the heart, lungs and skeletal muscles via the bloodstream or lymphatic circulation. In experimentally inoculated mice, larvae were found in the central nervous system (Sprent 1955; Matoff and Komandarev 1965; Sato et al. 2004) rarely if ever resulting in clinical symptoms/neurological disorders. To induce neurological manifestations by *B. transfuga* larva migrans, Papini and Casarosa (1994) inoculated mice with high doses of infectious eggs and observed neurological symptoms in 2.5% of mice inoculated with 3000 eggs, 6.7% at a dose of 4000 eggs and in 20% of mice inoculated with 50,000 eggs. Ocular larva migrans in mice after administration of

3500 infectious *B. transfuga* eggs was reported by Papini et al. (1996).

In contrast to previous experiments in mice, in which only isolated clinical symptoms or neurological disorders occured (Sprent 1955; Matoff and Komandarev 1965; Sato et al. 2004), the mice in our study showed clinical symptoms in most cases, regardless of the lower or higher infectious dose. However, the mice that received a higher dose of 2000 eggs were more clinically affected. The symptoms of dyspnoea and ascites corresponded to the histological findings in the lungs and liver. Some mice showed symptoms such as slowed movements on the left side and torticollis, suggestive of neurological disorders. Nevertheless, in most of them, with the exception of two cases, the larvae found in their brains did not trigger any tissue reaction in the brain. These results are in contradiction with previous studies in which higher infectious doses of 3000 to 50,000 eggs were required to induce neurological manifestation in mice (Papini and Casarosa 1994) and in which the larvae in mice brains were surrounded or embedded by granulomatous reactions in the brains of mice (Sato et al. 2004). The infective dose of 2000 eggs used by Sato et al. (2004) resulted in one case of constant circling and three cases of motor incoordination in the infected mice; only one case was fatal, all other mice recovered completely within a week. The host response to the migrating *Baylisascaris* larvae is thought to be critical for the development of clinical symptoms. In the case of encapsulation of migrating larvae in the brain, infected rodents did not show neurological symptoms, but non-immobilized larvae in the brain caused neurological manifestations (Sheppard and Kazacos 1997; Sato et al. 2004). We observed different outcomes in the clinical manifestation of infection in mice compared to previous studies (Papini and Casarosa 1994; Sato et al. 2004), which may be due to differences in the immune response and the ability to encapsulate migrating larvae between hosts. Symptomatic infection in aberrant hosts caused by larva migrans would lead to an increase in *Baylisascaris* transmission in the wild as infected host become weakened or die and are more easily preyed upon or captured (Page 2013).The target regions of 28S, ITS1 and 2 (ITSs) and *cox*1 and 2, which were tested by Testini et al. 2011, showed the highest interspecific difference with *B. procyonis* in the case of ITS1; 28S rDNA and *cox*1 sequences showed high intraspecific similarity and high interspecific variations compared to other *Baylisascaris* species available in GenBank; the *cox*2 sequence showed low similarity with *B. transfuga* sequences available in GenBank. Molecular analysis of five markers selected in our study confirmed morphological identification of the species as *B. transfuga*, but only on the basis of 28S, it was not possible to distinguish *B trasfuga* from *B. schroederi* and *B. ailuri*. Thus, among the markers tested, COI and ITSs seem to be suitable for the identification of *B. transfuga.*

B. transfuga appears to be a very thriving parasite species in areas inhabited by bears. Nevertheless, the transmission pattern and the role of the aberrant host under natural conditions are still unclear. While *B. transfuga* was considered to be of low pathogenicity to aberrant hosts (Sprent 1955; Matoff and Komandarev 1965; Sato et al. 2004), the results of our study revealing high pathogenicity to mice are contributing to the knowledge of the relationships between *B. transfuga* and its aberrant hosts.

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Author contribution David Modrý, Jana Juránková and Lada Hofmannová designed the study. Jana Juránková and Lada Hofmannová did the experimental part of the study. Lucia Frgelecová, Jana Juránková and Lada Hofmannová did histological examination. Lada Hofmannová photographed the histological sections. Lucia Frgelecová evaluated histopathological findings. Ondřej Daněk did molecular analyses. Jana Juránková wrote the manuscript and all the authors read and approved the final manuscript.

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Data availability All published sequential data are available in the GenBank database under defined accession numbers.

Code availability Geneious Prime software 2020.2.4.

Declarations

Ethics approval Protocol 10–2015 approved by the UVS and the Central Commission for Animal Welfare, Czech Republic.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

References

Bauer C (2013) Baylisascariosis – infections of animals and humans with "unusual" roundworms. Vet Parasitol 193:404–412. https://doi.org/10.1016/j.vetpar.2012.12.036

Bowles J, Blair D, McManus D (1992) Genetic variants within the genus *Echinococcus* identified by mitochondrial DNA sequencing. Mol Biochem Parasitol 54:165–173. https://doi.org/10.1016/0166-6851(92)90109-W

Bugmyrin SV, Spiridonov SE (2019) First record of natural *Baylisascaris transfuga* (Ascaridoidea, Nematoda) infection in wild rodents. Parasitology 146:1714–1718. https://doi.org/10.1017/S0031182019001239

Franssen F, Xie K, Sprong H, van der Giessen J (2013) Molecular analysis of *Baylisascaris columnaris* revealed mitochondrial and nuclear polymorphisms. Parasites Vectors 6:124. https://doi.org/10.1186/1756-3305-6-124

Hoberg EP, Burek-Huntington K, Beckmen K, Camp LE, Nadler SA (2018) Transuterine infection by *Baylisascaris transfuga*: neurological migration and fatal debilitation in sibling moose calves (*Alces alces gigas*) from Alaska. Int J Parasitol Parasites Wildl 7:280–288. https://doi.org/10.1016/j.ijppaw.2018.07.005

Matoff K, Komandarev S (1965) Comparative studies on the migration of the larvae of *Toxascaris leonina* and *Toxascaris transfuga*. Z Parazitenkd 25:538–555

Page LK (2013) Parasites and the conservation of small populations: the case of *Baylisascaris procyonis*. Int J Parasitol Parasites Wildl 2:203–210. https://doi.org/10.1016/j.ijppaw.2013.05.003

Papini R, Cavicchio P, Casarosa L (1993) Experimental infection in chickens with larvae of *Baylisascaris transfuga* (Nematoda: Ascaridoidea). Folia Parasit 40:141–143

Papini R, Casarosa L (1994) Observation on the infectivity of *Baylisascaris transfuga* eggs for mice. Vet Parasitol 51:283–288. https://doi.org/10.1016/0304-4017(94)90166-X

Papini R, Renzoni G, Lo Piccolo S, Casarosa L (1996) Ocular larva migrans and histopathological lesions in mice experimentally infected with *Baylisascaris transfuga* embryonated eggs. Vet Parasitol 61:315–320. https://doi.org/10.1016/0304-4017(95)00825-X

Sapp SGH, Gupta P, Martin MK, Murray MH, Niedringhaus KD, Pfaff MA, Yabsley MJ (2017) Beyond the raccoon roundworm: the natural history of non-raccoon *Baylisascaris* species in the New World. Int J Parasitol Parasites Wildl 6:85–99. https://doi.org/10.1016/j.ijppaw.2017.04.003

Sato H, Matsuo K, Osanai A, Kamiya H, Akao N, Owaki S, Furuoka H (2004) Larva migrans by *Baylisascaris transfuga*: fatal neurological diseases in Mongolian jirds, but not in mice. J Parasitol 90:774–781. https://doi.org/10.1645/GE-3330

Sheppard CH, Kazacos KR (1997) Susceptibility of *Peromyscus leucopus* and *Mus musculus* to infection with *Baylisascaris procyonis*. J Parasitol 83:1104–1111. https://doi.org/10.2307/3284370

Sprent JFA (1955) On the invasion of the central nervous system by nematodes II. Invasion of the nervous system in ascariasis. Parasitology 45:41–55

Štrkolcová G, Goldová M, Šnábel V, Špakulová M, Orosová T, Halán M, Mojžišová J (2018) A frequent roundworm *Baylisascaris transfuga* in overpopulated brown bears (*Ursus arctos*) in Slovakia: a problem worthy of attention. Acta Parasitol 63:167–174. https://doi.org/10.1515/ap-2018-0019

Testini G, Papini R, Lia RP, Parisi A, Dantas-Torres F, Traversa D, Otranto D (2011) New insight into the morphology, molecular characterization and identification of *Baylisascaris transfuga* (Ascaridida, Ascarididae). Vet Parasitol 175:97–102. https://doi.org/10.1016/j.vetpar.2010.09.017