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Baylisascaris sp. infection in a pet kinkajou *Potos flavus*

K. TAIRA[*1], Y. UNE[2], V. ŠNÁBEL[3], H. SUGIYAMA[4]

[1]Laboratory of Parasitology, School of Veterinary Medicine, Azabu University, 1-17-71 Fuchinobe, Chuo-ku, Sagamihara, Kanagawa 252-5201, Japan, E-mail: *taira@azabu-u.ac.jp*; [2]Laboratory of Pathology, School of Veterinary Medicine, Azabu University, 1-17-71 Fuchinobe, Chuo-ku, Sagamihara, Kanagawa 252-5201, Japan; [3]Institute of Parasitology, Slovak Academy of Sciences, 040 01 Košice, Slovakia; [4]Department of Parasitology, National Institute of Infectious Disease, 1-23-1 Toyama, Shinjuku, Tokyo 162-8640, Japan

Summary

The nematodes of genus *Baylisascaris* are common intestinal roundworms of carnivores such as raccoons, skunks, badgers, martens and bears. This report describes *Baylisascaris* sp. infection in a pet kinkajou *Potos flavus* imported into Japan from Guyana. Nematode eggs were detected in feces of the juvenile kinkajou in 2011 during a routine veterinary examination. A sequence analysis of the ITS2 nuclear target clustered the examined isolate with *B. procyonis* and *B. columnaris*, with 7.8 – 8.8 % base differences from these taxa. Eleven tandem G-A repeats identified in the polymorphic repetitive region further differentiate the kinkajou's roundworm from recognized *Baylisascaris* species. This classified the studied isolate as referring to *Baylisascaris* sp., with its precise species delineation remaining to be determined. Given that the *Baylisascaris* sp. from the kinkajou is genetically closely affiliated with *B. procyonis* having a serious disease-producing capacity, the report appeals for precautions in informing people to avoid transmission risk.

Keywords: *Baylisascaris* sp.; *Potos flavus*; ITS2; zoonosis

Introduction

The nematodes of genus *Baylisascaris* are intestinal roundworms of carnivores such as raccoons, skunks, badgers, martens and bears, and their larvae migrate in a range of small animal paratenic hosts serving as prey (Kazacos *et al*., 2001; Gavin *et al.*, 2005). The most pathogenic *Baylisascaris* species is *B. procyonis*, which is indigenous in North American raccoons (*Procyon lotor*) and has been demonstrated to occur in raccoons introduced around the world. The high prevalence of *B. procyonis* infection has been reported especially in wild raccoons in Germany and those kept in zoos in Japan (Miyashita, 1993; Sato *et al.*, 2001; Bauer, 2011). The larvae of these nematodes cause ocular and neural larva migrans in humans, and infection can potentially result in serious encephalitis, with permanent deficits or even death (Kazacos *et al.*, 2011).

The kinkajou *Potos flavus* has also been reported to be a definitive host of *B. procyonis,* which is identified based on morphological observations (Overstreet, 1970). In December 2011, a routine veterinary examination of a juvenile male pet kinkajou that had been caught in nature, imported from Guyana to Japan and purchased from an exotic pet shop in the same month, revealed ascarid eggs in the feces. The kinkajou was orally administrated once with an anthelmintic containing 72 mg of pyrantel, 75 mg of febantel and 25 mg of praziquantel (Drontal® Plus, Bayer). One day after the treatment, a nematode body fragment was expelled in the feces. No eggs had been detected in feces after the treatment.

This report describes finding of *Baylisascaris* sp. in a pet kinkajou in Japan, which was analyzed using the second internal transcribed spacer (ITS2) rDNA sequence, and outlines the potential risk of human infection with *Baylisascaris* sp. associated with the keeping of kinkajous as pets.

Materials and methods

Parasite material

A fragment of a nematode body measuring approximately 20 mm-long and 2 mm-wide was obtained from feces of a pet kinkajou kept in Kanagawa Prefecture, Japan. The samples were preserved in 5 ml of 10 % formalin solution for 2 days. No intact worm bodies or posterior or anterior ends of the worms were obtained.

Fecal examination

To detect parasite eggs, the fecal debris of the kinkajou (< 0.5 g) was examined by the flotation method using a saturated NaCl solution containing glucose (specific gravity of 1.27) (Roepstorff & Nansen, 1998). The dimensions of the obtained eggs (n = 20) were thereafter measured.

Molecular procedures

The nematode tissue sample was washed with TE buffer (pH 8.0) solution and then incubated in the buffer overnight. Genomic DNA was extracted from the tissue using a QIAamp DNA Mini Kit (Qiagen, Tokyo, Japan) according to the manufacturer's instructions, with several modifications (Šnábel *et al.*, 2012). The DNA extract was used as template for amplification of the second internal transcribed spacer (ITS2) region of ribosomal DNA by PCR. The forward primer LC1 (5'-CGACTATCGATGAAGAA CGCAGC-3') and the reverse primer HC2 (5'-ATATGCT TAAGTTCAGCGGG-3'), which corresponded to the conserved 3' and 5' ends of the 5.8S-ITS2-28S regions, were used. Primers were formerly designed by Ellis *et al.*, (1986) and Qu *et al*. (1988), and were simultaneously used in a phylogenetic study on schistosomes undertaken by Despres *et al*. (1992). The PCR procedure was performed using the following protocol: 94 °C for 12 min (polymerase activation), followed by 30 cycles of denaturation at 94 °C for 30 sec, annealing at 58 °C for 45 sec, extension at 72 °C for 45 sec, and a final extension step of 72 °C for 7 min. The amplicons (2 µl) were examined by 2 % ethidium bromide-stained agarose gel electrophoresis to assess the amplification efficiency. The selected PCR products were then purified and sequenced in both directions using an ABI3730XL automated sequencer (Life Technologies, CA).

To seek nematodes with the closest genetic relatedness to the examined sample, the sequence homology was searched using the Basic Local Alignment Search Tool (BLAST 2.2.22) tool. The 5' and 3' ends of the ITS2 sequence commonly determined for *B. procyonis* were verified by comparison with the consensus sequences for related ascarid isolates stored in GenBank, namely *Baylisascaris procyonis* (GenBank™ accession number JQ4036151), *B. columnaris* genotype 1 (KC543487), *B. columnaris* genotype 2 (KC543486), *B. transfuga* (HM594951), *B. schroederi* (JN210912), *Ascaris suum* (AB571302), *A. lumbricoides* (AB571298), *Parascaris equorum* (JN617987), *T. canis* (JF837169), *Toxocara vitulorum* (FJ418784), *T. cati* (AB571303), and *Toxascaris leonina* (JF837174).

These reference sequences were then aligned with the sequence obtained in the present study using ClustalW software (Thompson *et al.*, 1994). To indicate the phylogenetic position of nematode from the pet kinkajou and to evaluate evolutionary relationships within the group of ascarid nematodes, the compromised sequences trimming to equal lengths were subjected to clustering analysis using the neighbor-joining (NJ) and maximum likelihood (ML) clustering methods with the Kimura 2-parameter calculated for a distance matrix. *Caenorhabditis elegans* (JN636101) was used as an out-group. Bootstrap analysis with 1,000 replicates was performed to assess tree robustness using the MEGA 5 program (Tamura *et al*., 2011). The sequence of the kinkajou isolate was deposited in GenBank with the accession number KF680774.

Results

Eggs detected in the feces of a pet kinkajou were ellipsoidal in shape, brown in color, and contained large unicellular embryo with a thick shell and roughly granular surface (Fig. 1). The eggs measured 76.8 ± 2.26 µm (n = 20, mean ± SD; range: 73 – 81 µm) in the long axis and 64.4 ± 1.02 µm (range: 62.9-66.5 µm) in the transverse axis.

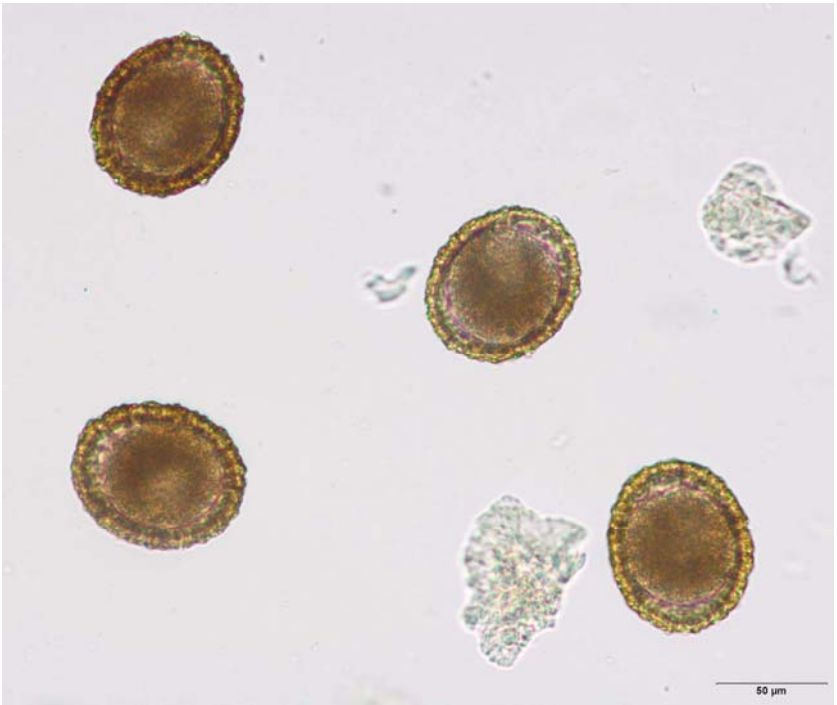

Fig. 1. Nematode eggs detected in feces of a pet kinkajou by the flotation method using a saturated NaCl solution containing glucose (1.27 SG); Eggs measured 76.8 ± 2.26 µm (n = 20, mean ± SD; range: 73 – 81 µm) in the long axis and 64.4 ± 1.02 µm (range: 62.9 – 66.5 µm) in the transverse axis

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JP1             TGCGATAAATAGTGCGAATTGCAGACACATTGAGCACTAAAAATTCGAAC 50
BP (JQ403615)   .................................................. 50
BC1(KC543487)   .................................................. 50
BC2(KC543486)   .................................................. 50

JP1             GCACATTGCGCCATCGGGTTCATTCCCGTTGGCACGTCTGGCTGAGGGTT 100
BP (JQ403615)   .T................................................ 100
BC1(KC543487)   .T................................................ 100
BC2(KC543486)   .T................................................ 100

JP1             GAAATATCGTAAGAATTGCCATTTATGAATTTTCAATATGGCATATTCGA 150
BP (JQ403615)   ..............G.....................C.........C... 150
BC1(KC543487)   ..............G.....................C.........C... 150
BC2(KC543486)   ..............G.....................C.........C... 150

JP1             TAAGCTATGGTGGTAGACGAATAAAAGAAGTACTATCGTACCTTCTTCAG 200
BP (JQ403615)   .........A............................A............ 200
BC1(KC543487)   .........A............................A............ 200
BC2(KC543486)   .........A............................A............ 200

JP1             CATATATGATGCAATAACTCGTTCTCATTTGCTTCGACGAGCTCAGAGAG 250
BP (JQ403615)   ............................C....A.T...A.G...... 250
BC1(KC543487)   ............................C....A.T...A.G...... 250
BC2(KC543486)   ............................C....A.T...A.G...... 250

JP1             AGAGAGAGAGAGAGAGAAAGAGAAAGAAAGAAAGAAAGAGAAAGAATATA 300
BP (JQ403615)   AGAGAGAGAGAGA----------------------AAGAGAAAGAATATA 278
BC1(KC543487)   AGAGAGAGA-------------------------AAGAGAAAGAATATA 274
BC2(KC434486)   AGAGAGA---------------------------AAGAGAAAGAATATA 272

JP1             TGCATCAAGAAATTATCGTGTCGCTCTTAAAAATCGATTCCAGCGTATAT 350
BP (JQ403615)   ................................G................. 328
BC1(KC543487)   ................................G................. 324
BC2(KC543486)   ................................G................. 322

JP1             TGTTATGGATCTAGCAATATGCCATAGTTGGAAAGAAAGATAGGCGATAA 400
BP (JQ403615)   .................................................. 378
BC1(KC543487)   .................................................. 374
BC2(KC543486)   .................................................. 372

JP1             TGATGCATATAAAGGATTTTTTGACCTCAGCTCA 434
BP (JQ403615)   ................................... 412
BC1(KC543487)   ................................... 408
BC2(KC543486)   ................................... 406
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Fig. 2. Polymorphic sites in ITS2 sequences (434 bp) of JP1 kinkajou isolate related to *Baylisascaris procyonis* (BP); *B. columnaris*, genotype 1 (BC1); *B. columnaris*, genotype 2 (BC2). Dots indicate identity with reference (JP1) sequence

The PCR product in the interpretable length of 434 bp was yielded by amplification of the rDNA region spanning the 3' end of the 5.8S, ITS2, and 5' end of 28S rDNA from the worm fragment. BLAST homology analysis revealed that the closest phylogenetic relatives to the nematode in the present study; herein denoted as JP1 isolate, are *B. procyonis* and *B. columnaris* among present GenBank data. *B. procyonis* isolate from raccoon in Norway (JQ403615) and two genotypes of *B. columnaris* expelled from skunks in Netherlands (KC543486, KC543487) were taken as references for analyses of variations in nucleotide composition. Pairwise comparison showed that the JP1 isolate differed in 7.8 % of bases from *B. procyonis* by manifesting 22 insertions and 12 nucleotide substitutions, and in 8.3 % – 8.8 % of bases from the two *B. columnaris* genotypes by manifesting 24 – 26 insertions and 12 nucleotide substitutions (Fig. 2).

A substantial part of variation differentiating the kinkajou

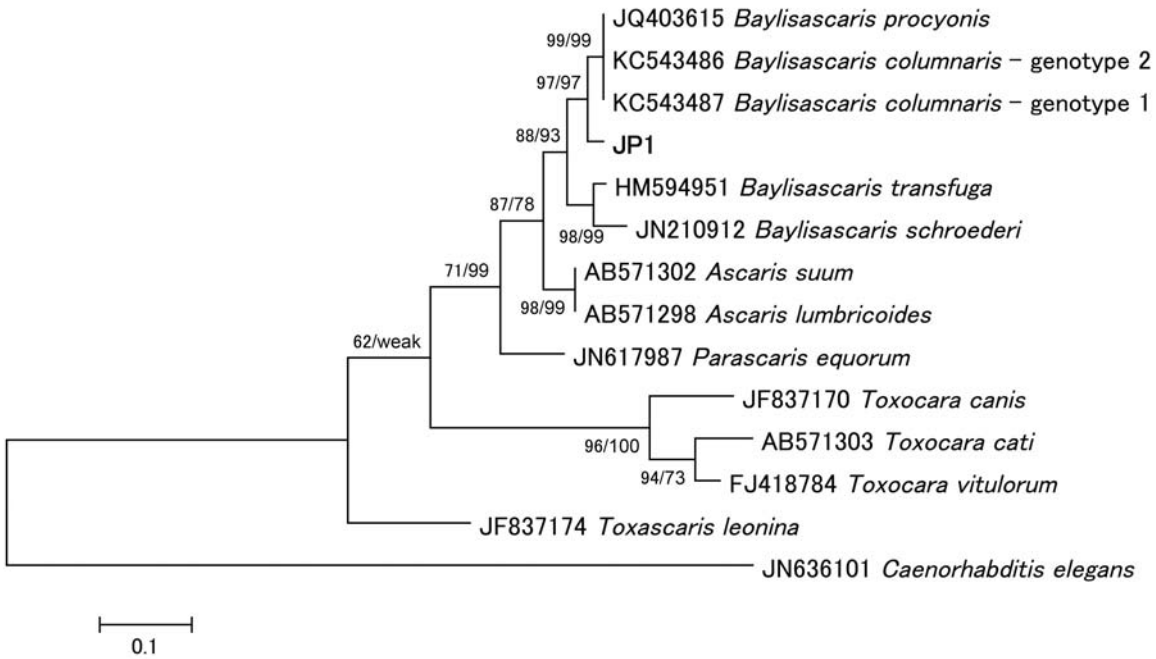

Fig. 3. Dendrogram constructed by neighbor-joining (NJ) and maximum likelihood (ML) methods based on ITS2 nuclear sequences of *Baylisascaris* and related ascarid species; JP1 isolate was examined in the present study; Numbers at the nodes of branches refer to bootstrap values (1,000 pseudoreplications) derived from NJ (first value) and ML analyses. *Weak* bootstrap support was indicated at values < 60%

isolate JP1 from the related species is attributed to 22 – 26 insertions from positions 264 upwards, partially consisting of G-A tandem repeats, the number of which consistently distinguishes *Baylisascaris* species. For nucleotide substitutions, the most polymorphic region for JP1 isolate, comprising 5 of 12 base exchanges, was located between positions 231 – 244 of ITS2 (Fig. 2). Among recorded nucleotide exchanges relative to *B. procyonis*, 9 were transitions and 3 transversions. Resulting transition/transversion (ts/tv) ratio 3:1 for these taxa is generally assumed for closely related organisms.

Phylogenetic trees constructed by NL and ML analyses of ITS2 sequences of the 11 ascarid species revealed the similar topology, with two large clades separating Ascarididae and Toxocaridae isolates. The analyzed JP1 isolate was located in a well-supported subclade (bootstrap 88 % in NJ, 93 % in ML) consisting of *Baylisascaris* representatives, with the two clusters being inferred for this genus. The JP1 isolate has grouped together with *B. procyonis* and *B. columnaris*, with some discrimination from these taxa (Fig. 3). From remaining *Baylisascaris* species, *B. transfuga* and *B. schroederi* located in second cluster, the JP1 isolate differed in 14.3 % and 15.4 % of ITS bases, respectively.

Discussion

In the present study, a peculiar genetic composition of a *Baylisascaris* isolate from a pet kinkajou imported to Japan from South America, consistently differing from the closest *B. procyonis* with public health relevance, was recorded.

The ascarid eggs detected in feces from the kinkajou were morphologically similar to those of *B. procyonis* (raccoon roundworm), with eggs measuring 63-88 µm (mean: 68-76 µm) by 50 – 70 µm (mean: 55-61 µm), and *B. columnaris* (skunk roundworm) with eggs measuring 72.5 ± 4.1 µm by 63.2 ± 7.5 µm (Kazacos *et al.*, 2011; Franssen *et al.*, 2013). Slightly closer values with regard to the JP1 isolate were thus associated with *B. columnaris*, particularly in the length of transverse axis (64.4 ± 1.02 µm in JP1 isolate). Nevertheless, morphometric identification in *Baylisascaris* is often hampered by diversity in size and developmental stages that makes the unequivocal recognition of several species including *B. procyonis* and *B. columnaris* problematic (Berry, 1985; Franssen *et al*., 2013).

The nuclear rDNA internal transcribed spacer is extensively being used for phylogenetic reconstruction and distinguishing between closely related species, including ascarids (Zhu *et al.*, 2001; Pawar *et al.*, 2012). In the present study, a unique divergence pattern was derived from the sequences of the ITS2 region for the kinkajou isolate suggesting that the nematode could not be entirely allocated to the closest recognized *Baylisascaris* species. Molecular prospecting as a tool for search and evaluation of the existence of separate taxa within the morphospecies has been often addressed to more clarify systematics in ascarid nematodes (Nadler & Huspeth, 2000; Derycke *et al.*, 2010). As Blouin (2002) summarized, the level of fixed sequence difference in ITS between closely related nematodes is relatively frequently ≤ 1 %, unlike mitochondrial DNA, in which helminth species typically differ in at least 10 % of sequences (Vilas *et al.*, 2005). The 7.8 – 8.8 %

range of nucleotide difference between the JP1 isolate and the closest retrieved sequences of *B. procyonis* and *B. columnaris* in ITS2 therefore classified the isolate from the pet kinkajou as referring to *Baylisascaris* sp., with its precise species categorization remaining to be determined.

Another question emerges whether or not the present isolate pertains to any described *Baylisascaris* spp., for which sequencing data are not yet available in GenBank. The genus currently contains eight recognized species (Kazakos, 2013). From these, apart from 4 species with available nuclear sequences, transmissions of *B. melis* linked to badgers absent in South America, and *B. tasmaniensis* with marsupial carnivore hosts endemic for Tasmania, exclude these taxa as plausible for infesting kinkajou in Guyana. From remaining species, *B. laevis*, morphologically similar to *B. columnaris* and *B. procyonis* (Berry, 1985; Anderson, 2000), occurring in large rodents as marmots and ground squirrels, and *B. devosi* occurring in martens and fishers, are transmissible in South American wildlife. A type material from these species is therefore needed to be subjected to further sequence analyses to better clarify the taxonomic issue in the JP1 isolate.

Vilas *et al*. (2005) appointed that because the ITS is a non-coding sequence, frequent deletions and insertions are often present making alignment more complicated. This was also the case of ribosomal data gathered in this study, with the preponderance of insertions in the examined isolate compared to the present *Baylisascaris* sequences. A varying number of G-A tandem repeats can be used as discriminative marker for *Baylisascaris* spp. in the specific zone of the ITS2 region (positions 246 and further). Nine G-A tandem repeats were identified for *B. procyonis*, eight for *B. transfuga*, seven and six for the two *B. columnaris* genotypes, and one for *B. schroederi* that likely represents the ancestral state of the given DNA region (Testini *et al.*, 2011; Lin *et al.*, 2012; Franssen *et al.*, 2013). For the JP1 isolate, 11 tandem contiguous G-A repeats were recorded in the respective locations. Two of them along with the additional interspersed motifs with G, A bases have constituted 22-26 insertions compared to *B. procyonis* and *B. columnaris*, indicating a tendency towards lengthening the ITS2 region. The proposed mechanisms of evolution of repetitive sequences include both intra- or interstrand recombinational effects or mechanisms involving failures in the DNA replication (Platas *et al.*, 2001). Mutational changes can create new motifs that may be propagated by additional slipped-strand mispairing (SSM) events. This was the plausible scenario for amplifying G-A repetitions in *Baylisascaris* spp., which formerly likely arose by chance in ITS2 and could have been further generated by SSM events into longer repeats.

Based on the gathered data, it is likely that the peculiar *Baylisascaris* species circulating in Guyanese zoofauna has been translocated to Japan via the infected kinkajou. Nevertheless, further characterization of *Baylisascaris* isolates from other kinkajou hosts is required, with particular emphasis on adult morphology and the pathogenicity of migrating larvae in paratenic hosts. In addition, to address potential limitations of single locus analyses, both nuclear and mtDNA regions will be targeted in molecular assays of *Baylisascaris* sp. to facilitate species delineation.

Till now, there are over 20 documented cases of ocular larva migrans and severe or even fatal neurological disturbances in humans implied by *B. procyonis* (Galvin *et al.*, 2005; Bauer, 2013). Zoonotic potential of *B. columnaris* is not yet known as serological assays do not discriminate between *Baylisascaris* species (Dangoudoubiyam et al., 2011). Experimental infections showed that *B. procyonis* is more pathogenic to mice than *B. columnaris* owing to faster growth to 1 mm size, which correlates with the first appearance of nervous symptoms (Tiner, 1953; Sheppard & Kazakos, 1997). However, both species are along with *B. melis* (principal host badger) regarded as the most dangerous *Baylisascaris* members because of their serious disease-producing capabilities (Kazakos, 2001).

Given that the *Baylisascaris* sp. from the kinkajou is genetically closely affiliated with *B. procyonis*, the potential risk of human infection with *Baylisascaris* from this host may be considerable. Therefore, transmission-based precautions are needed to prevent human infections of pet kinkajous. It is strongly recommended that owners should take their kinkajous regularly to veterinarian for periodic fecal examination.

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References

ANDERSON, R. C. (2000): Nematode parasites of vertebrates: Their development and transmission, 2nd ed. Wallingford, UK: CAB International, 650 pp.

BAUER, C. (2011): Baylisascariosis (*Baylisascaris procyonis*) – a rare parasitic zoonosis in Europe. *Berl. Munch. Tierarztl. Wochenschr.,* 124: 465 – 472 (In German)

BAUER, C. (2013): Baylisascariosis – Infections of animals and humans with 'unusual' roundworms. *Vet. Parasitol.,* 193: 404 – 412. DOI: 10.1016/ j.vetpar.2012.12.036

BERRY, J. F. (1985): Phylogenetic relationship between *Baylisascaris* spp. Sprent, 1968 (Nematoda: Ascarididae) from skunks, raccoons and groundhogs in southern Ontario. M. S Thesis. University of Guelph, Guelph, Ontario, Canada, 99 pp.

BLOUIN, M. S. (2002): Molecular prospecting for cryptic species of nematodes: mitochondrial DNA versus internal transcribed spacer. *Int. J. Parasitol.,* 32: 527 – 531

DANGOUDOUBIYAM, S., VEMULAPALLI, R., NDAO, M., KAZACOS, K. R. (2011): Recombinant antigen-based enzyme-linked immunosorbent assay for diagnosis of

Baylisascaris procyonis larva migrans. *Clin. Vaccine Immunol.,* 18: 1650 – 1655. DOI: 10.1128/CVI.00083-11
DERYCKE, S., DE LEY, P., DE LEY, I. T., HOLOVACHOV, O., RIGAUX, A., MOENS, T. (2010): Linking DNA sequences to morphology: cryptic diversity and population genetic structure in the marine nematode *Thoracostoma trachygaster* (Nematoda, Leptosomatidae). *Zool. Scr.*, 39: 276 – 289. DOI: 10.1111/j.1463-6409.2009.00420.x
DESPRES, L., IMBERT-ESTABLET, D., COMBES, C., BONHOMME, F. (1992): Molecular evidence linking hominid evolution to recent radiation of schistosomes (Platyhelminthes: Trematoda). *Mol. Phylogenet. Evol.,* 1: 295 – 304
ELLIS, R. E., SULSTON, J. E., COULSON, A. R. (1986): The rDNA of *C. elegans*: sequence and structure. *Nucleic Acids Res.,* 14: 2345 – 2364
FRANSSEN, F., XIE, K., SPRONG, H., VAN DER GIESSEN, J. (2013): Molecular analysis of *Baylisascaris columnaris* revealed mitochondrial and nuclear polymorphisms. *Parasit. Vectors*, 6: 124. DOI: 10.1186/1756-3305-6-124
GAVIN, P. J., KAZACOS, K. R., SHULMAN, S. T. (2005): Baylisascariasis. *Clin. Microbiol. Rev.* 18: 703 – 718
KAZACOS, K. R., KILBANE, T. P., ZIMMERMAN, K. D., CHAVEZ-LINDELL, T., PARMAN, B., CARPENTER, L. R., GREEN, A. L., MANN, P. M., MURPHY, T. W., BERTUCCI, B., GRAY, A. C., GOLDSMITH, T. L., CUNNINGHAM, M., STANEK, D. R., BLACKMORE, C., YABSLEY, M. J., MONTGOMERY, S. P., BOSSERMAN, E. (2011): Raccoon roundworms in pet kinkajous--three states, 1999 and 2010. *MMWR Morb. Mortal. Wkly. Rep.,* 60: 302 – 305
KAZACOS, K. R. (2001): *Baylisascaris procyonis* and related species. In: SAMUEL, W. M., PYBUS, M. J., KOCAN, A. A. (Eds) Parasitic diseases of wild animals., 2nd ed. Iowa State University Press, London, pp. 301-341
MIYASHITA, M. (1993): Prevalence of *Baylisascaris procyonis* in raccoons in Japan and experimental infections of the worm to laboratory animals. *J. Urban Living Health Assoc.*, 37: 137 – 151 (In Japanese)
LIN, Q., LI, H. M., GAO, M., WANG, X. Y., REN, W. X., CONG, M. M., TAN, X. C., CHEN, C. X., YU, S. K., ZHAO, G. H. (2012): Characterization of *Baylisascaris schroederi* from Qinling subspecies of giant panda in China by the first internal transcribed spacer (ITS-1) of nuclear ribosomal DNA. *Parasitol. Res.,* 110: 1297 – 1303
NADLER, S. A., HUDSPETH, D. S. (2000): Phylogeny of the Ascaridoidea (Nematoda: Ascaridida) based on three genes and morphology: hypotheses of structural and sequence evolution. *J. Parasitol.,* 86: 380 – 393
OVERSTREET, B. M. (1970): *Baylisascaris procyonis* (Stefanski and Zarnowski, 1951) from the kinkajou, *Potos flavus*, in Colombia. *Proc. Helminthol. Soc.,* 32: 192 – 195
PAWAR, R. M., LAKSHMIKANTAN, U., HASAN, S., POORNACHANDAR, A., SHIVAJI, S. (2012): Detection and molecular characterization of ascarid nematode infection (*Toxascaris leonina* and *Toxocara cati*) in captive Asiatic lions (*Panthera leo persica*). *Acta Parasitol.,* 57: 67 – 73. DOI: 10.2478/s11686-012-0012-y
PLATAS, G., ACERO, J., BORKOWSKI, J. A., GONZÁLEZ, V., PORTAL, M. A., RUBIO, V., SÁNCHEZ-BALLESTEROS, J., SALAZAR, O., PELÁEZ, F. (2001): Presence of a simple tandem repeat in the ITS1 region of the Xylariales. *Curr. Microbiol.,* 43: 43 – 50
QU, L. H., NICOLOSO, M., BACHELLERIE, J. P. (1988): Phylogenetic calibration of the 5′ terminal domain of large rRNA achieved by determining twenty eucaryotic sequences. *J. Mol. Evol.,* 28: 113 – 124
ROEPSTORFF, A., NANSEN, P. (1998): The epidemiology, diagnosis and control of helminth parasites of swine. FAO Animal Health Manual No. 3, FAO, Rome, Italy.
SATO, H., FURUOKA, H., KAMIYA, H. (2001): First outbreak of *Baylisascaris procyonis* larva migrans in rabbits in Japan. *Parasitol. Int.*, 51: 105 – 108
SHEPPARD, C. H., KAZACOS, K. R. (1997): Susceptibility of *Peromyscus leucopus* and *Mus musculus* to infection with *Baylisascaris procyonis. J. Parasitol.,* 83: 1104 – 1111
ŠNÁBEL, V., TAIRA, K., CAVALLERO, S., D'AMELIO, S., RUDOHRADSKÁ, P., SAITOH, Y. (2012): Genetic structure of *Ascaris* roundworm in Japan and patterns of its geographical variation. *Jpn. J. Infect. Dis.,* 65: 179 – 183
TAMURA, K., PETERSON, D., PETERSON N., STECHER, G., NEI, M. (2011): MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Mol. Biol. Evol.,* 28: 2731 – 2739. DOI: 10.1093/molbev/msr121
TESTINI, G., PAPINI, R., LIA, R. P., PARISI, A., DANTAS-TORRES, F., TRAVERSA, D., OTRANTO, D. (2011): New insights into the morphology, molecular characterization and identification of *Baylisascaris transfuga* (Ascaridida, Ascarididae). *Vet. Parasitol.,* 175: 97 – 102. DOI: 10.1016/j.vetpar.2010.09.017
THOMPSON, J. D., HIGGINS, D. G., GIBSON, T. J. (1994): Clustal W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.,* 22: 4673 – 4680
VILAS, R., CRISCIONE, C. D., BLOUIN, M. S. (2005): A comparison between mitochondrial DNA and the ribosomal internal transcribed regions in prospecting for cryptic species of platyhelminth parasites. *Parasitology,* 131: 839 – 846
ZHU, X. Q., GASSER, R. B., CHILTON, N. B., JACOBS, D. E. (2001): Molecular approaches for studying ascaridoid nematodes with zoonotic potential, with an emphasis on *Toxocara* species. *J. Helminthol*., 101 – 108

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