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Transuterine infection by *Baylisascaris transfuga*: Neurological migration and fatal debilitation in sibling moose calves (*Alces alces gigas*) from Alaska

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ABSTRACT

Larval *Baylisascaris* nematodes (L3), resulting from transuterine infection and neural migration, were discovered in the cerebrum of sibling moose calves (*Alces alces gigas*) near 1–3 days in age from Alaska. We provide the first definitive identification, linking morphology, biogeography, and molecular phylogenetics, of *Baylisascaris transfuga* in naturally infected ungulates. Life history and involvement of paratenic hosts across a broader assemblage of mammals, from rodents to ungulates, in the transmission of *B. transfuga* remains undefined. Neural infections, debilitating young moose, may seasonally predispose calves to predation by brown bears, facilitating transmission to definitive hosts. Discovery of fatal neurological infections by L3 of *B. transfuga* in mammalian hosts serves to demonstrate the potential for zoonotic infection, as widely established for *B. procyonis,* in other regions and where raccoon definitive hosts are abundant. In zones of sympatry for multi-species assemblages of *Baylisascaris* across the Holarctic region presumptive identification of *B. procyonis* in cases of neurological larval migrans must be considered with caution. Diagnostics in neural and somatic larval migrans involving species of *Baylisascaris* in mammalian and other vertebrate hosts should include molecular-based and authoritative identification established in a phylogenetic context.

1. Introduction

The significance of infections attributed to ascaridoid nematodes among potential intermediate or paratenic hosts is in a propensity for somatic, visceral and neural migration by infective third stage larvae (L3) leading to significant disease and mortality (e.g., Sprent, 1952; Tiner, 1953; Kazacos, 1997, 2001; 2016; Papini et al., 1996; Gavin et al., 2005). Among this assemblage of nematodes neural migration is perhaps best exemplified by L3 among species of *Baylisascaris* Sprent, 1968 and especially *Baylisascaris procyonis* (Stefanski and Zarnowski, 1951). A large nematode of raccoon [*Procyon lotor* (Linnaeus)] endemic in temperate latitudes of North America, *B. procyonis* has also been introduced and established in central Europe and Japan (Okulewicz and Buńkowska, 2009; Beltran-Beck et al., 2012; Robertson et al., 2013; Kazacos, 2016). Although primarily seen in raccoon definitive hosts as adult parasites, the L3 of *B. procyonis* have a broad range of paratenic hosts involved in transmission. Larvae have been documented among a remarkably diverse assemblage of mammals and birds, including reports of significant and devastating zoonotic infections in people (e.g., Kazacos, 1986, 2001; 2016; Anderson, 2000; Gavin et al., 2005; Bauer, 2013; Sapp et al., 2017). In the temperate/boreal zone of North America and areas where *Baylisascaris* has been introduced in Europe or Asia, presumptive diagnosis in cases of neural larval migrans among paratenic hosts has been *B. procyonis* or less often *B. columnaris* (Leidy, 1856). These diagnoses, in the absence of verified vouchers, have been common even in zones of expected sympatry with other congeneric species (reviewed in Kazacos, 2001). The role of true intermediate or paratenic hosts in development and transmission among species of these ascaridoid nematodes in mammalian hosts remains to be clearly defined (Sapp et al., 2017); we adopt the usage of paratenic host in the

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context of our observations and the following report.

Across the Holarctic and into the Neotropical region, species of *Baylisascaris* are generally typical parasites of medium to large carnivoran definitive hosts including the giant panda with a single species occurring in large rodents (Sprent, 1968; Kazacos, 2001; Zhang et al., 2011; Bauer, 2013). Globally 11 species are recognized, including 4 that are endemic to the Western Hemisphere, 4 species distributed across the Holarctic, 2 endemic to Asia and a single species from Tasmania (Bauer, 2013; Mata et al., 2016; Sapp et al., 2017). Species in carnivores at higher boreal and subarctic latitudes, and also more widely distributed in North America, are represented by *B. transfuga* (Rudolphi, 1819) in brown bear (*Ursus arctos* Linnaeus) and black bear (*U. americanus* Pallas) and *B. devosi* (Sprent, 1952) in medium to large mustelids including weasels and ermine (species of *Mustela* Linnaeus), marten [*Martes americana* (Turton) and *M. caurina* (Merriam)], fisher [*Pekania pennanti* (Erxleben)] and wolverine (*Gulo gulo* Linnaeus) (e.g., Rausch, 1959; Choquette et al., 1969; Hoberg et al., 1990; Borka-Vitális et al., 2017; Sapp et al., 2017). Additional species, including *B. procyonis* in raccoons, *B. columnaris* in skunks (species of *Mephitis* É. Geoffroy Saint-Hilaire and Cuvier and *Spilogale* Gray) and possibly *B. melis* (Gedoelst, 1920) in badgers [*Taxidea taxus* (Schreber)] are currently restricted to more southern, temperate latitudes. The entire assemblage of 4–5 species, however could be in sympatry at some localities in North America (e.g., Pence et al., 1983; Kazacos, 2001; Sapp et al., 2017).

We document and report the occurrence of fatal larval infections involving neural migration by a species of *Baylisascaris* in sibling moose calves (*Alces alces gigas* Miller) from Alaska. Definitive morphological and molecular identification confirmed infection by *Baylisascaris* and in conjunction with recent phylogenetic analyses among species of the genus provided a species level diagnosis (Camp et al. submitted). Neural infections by L3 of *Baylisascaris* in ungulates have been rarely reported and none have been definitively identified at the species level (Anderson, 1999; Kazacos, 2016; Sapp et al., 2017). Further, the current infections clearly represent the outcome of transuterine migration and intrauterine infection. We explore the pathology involved in these cases and the implications for life history patterns and transmission of *Baylisascaris* among bears and moose in Alaska.

2. Materials and methods-

2.1. Brief history

Twin moose calves from Kincaid Park, a municipal park in Anchorage, Alaska (ca., 61.1541° N and 150.0167° W), estimated to be 1–3 days in age, were orphaned on 23 May 2011 when their dam was shot for aggressive behavior. The calves were subsequently transported to a permitted holding facility maintained by the Alaska Moose Federation, at Palmer, AK. On arrival both calves were wobbly and with wet umbilical cords, indicative of recent birth. The male calf (Alaska Department of Fish and Game, necropsy ID 2011-067 Pathology ID VII-126) was consistently weak and apparently could not suckle or swallow properly, was euthanized on 30 May 2011 and necropsied 24 h later. The female sibling was more vigorous, but later broke her leg, and was euthanized and necropsied on 19 August 2011(Pathology ID VII-201-ADFG OMC ID Tag 56). At necropsy, a panel of tissues was collected for histological evaluation from each calf with specimens fixed in 10% neutral buffered formalin. Intact tissue from the cerebellum and cerebrum of the female calf was also cryo-archived at minus 20 C.

2.2. Histology of the nervous system

A suite of tissues including brains were collected for histology and preserved in 10% neutral buffered formalin, processed at Histology Consulting Services (Pullman, Washington), and slides analyzed by a pathologist (KBH). Briefly, paraffin-embedded sections were cut to 5 μm, stained with hematoxylin and eosin and permanently mounted.

2.3. Recovery of intact L3 from brain specimen

A frozen, unfixed, tissue sample of several grams from the cerebellum of the infected female calf was received at the US National Parasite Collection in April 2012 (EPH). Tissue was examined in detail initially by compression and transmitted illumination at magnifications to 60×, and secondarily by physical maceration in saline and sieving through a 200 mesh (75 μm) screen. A single larva was recovered from macerated tissue, preserved in 95% ethanol and held at minus 80C for later molecular characterization. Further microscopy of this larval specimen was not conducted given the potential for damage or loss which would have jeopardized attempts for a definitive species-level identification based on DNA sequence comparisons.

2.4. DNA extraction and PCR

A presumptive L3 of *Baylisascaris* recovered from the brain tissue was received in the Department of Entomology and Nematology at the University of California, Davis (SN and LEC). This larval specimen was destructively sampled. DNA from a single *Baylisascaris* L3 was prepared using the Epicentre MasterPure Complete DNA Purification Kit (Epicentre Biotechnologies, Madison, Wisconsin). Regions of two mitochondrial genes (12S ribosomal DNA, cytochrome *c* oxidase subunit 2 [*cox2*]), and two nuclear rDNA genes (large-subunit [28S] and the internal transcribed spacers/5.8S gene [ITS-1, 5.8S, ITS-2, abbreviated ITS]) were amplified by PCR. Primers 505 and 506 were used to amplify the 12S region; primers 210 and 211 were used to amplify the *cox2* region. Primers 391 and 501 were used to amplify the 5' end of the 28S rDNA; and primers 521 and 94 were used to amplify the ITS region. Primer sequences for all genes used for PCR or sequencing are described in Table 1. Other species of *Baylisascaris* and individuals used in the phylogenetic analyses (sequences obtained from GenBank), DNA extraction methods and other procedures are provided in Camp et al. (submitted).

For the *Baylisascaris* larva, 25 μl polymerase chain reactions contained 200 μM deoxynucleoside triphosphates, 0.5 units of KOD XL polymerase (EMD Millipore, Billerica, MA), 0.5 μM of each primer, and 2.5 μl of DNA template. For 12S and LSU amplifications, cycling parameters followed Nadler et al. (2006). For *cox2*, cycling parameters followed Nadler and Hudspeth (2000). For ITS, cycling parameters included a 4 min denaturation at 94 °C, then 35 cycles of 94 °C for 30 s, 56 °C for 30 s, 72 °C for 1 min, and a final extension of 7 min at 72 °C.

PCR products were enzymatically treated for sequencing with exonuclease I and shrimp alkaline phosphatase (USB Affymetrix Pre-sequencing kit, USA) and directly sequenced using an ABI 3730 DNA Sequencer (Applied Biosystems, Thermo Fisher Scientific) with the PCR primers (12S, *cox2*), or both PCR primers and internal primers (ITS, 28S, Table 1).

Sequence contigs were assembled using CodonCode Aligner (version 5.1.5, CodonCode Corporation, Centerville, Massachusetts) and Phred base calling. All sequences were double-stranded for verification. Site polymorphisms were recorded only when both alternative nucleotide peaks were present in all sequencing reactions representing both DNA strands. If the heights of the alternative nucleotide peaks at polymorphic sites were not equal, the height of the minor peak was required to significantly exceed background terminations and comprise ≥25% of the major peak to be scored as a polymorphism. GenBank accession numbers for all analyzed sequences are provided in Table 2.

2.5. Phylogenetic analysis

Multiple Alignment: For non-coding genes (12S, 28S, ITS) sequences for each species or geographic isolate were aligned using ProAlign v0.5a0 (Löytynoja and Milinkovitch, 2003). Nucleotide sequences of