Wacky Work on Worms in Wolverines

The identification of *Baylisascaris devosi* parasite

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Abstract

Parasitic roundworms (nematodes) and tapeworms (cestodes) are commonly found in wildlife. They often have complex life cycles with intermediate and definitive hosts. Very little is known about intestinal worms in wolverines. Because certain species of parasitic worms can be harmful to animals as well as humans (zoonotic parasites), it is especially important to be able to identify these parasites properly. The objective of this study was to identify parasitic worms in wolverines by morphological and molecular techniques. We examined intestines from 9 wolverines and found 59 parasitic worms in total. Of these parasites, 58 were of the genus *Baylisascaris* (roundworm) and one *Taenia* (tapeworm) as determined by morphological identification. We used a polymerase chain reaction method on 10 samples (9 *Baylisascaris* and one *Taenia*) to identify what species they belonged to. After comparison with known genetic sequences in the GenBank™, we concluded that we found a genetically undocumented species of *Baylisascaris*. This study is important from a basic scientific perspective because it adds to our knowledge of parasitic diseases in Canadian wildlife. It is also important because identification by molecular means offers a quick, non-invasive molecular method to identify this parasite based on worm eggs in wolverine fecal samples, which has important implications for wildlife conservation. This study also has implications for diagnosis of human infections. The closest known relative to *B. devosi* is *B. columnaris from skunks,* which has the potential to be infectious to humans (zoonotic) similar to its relative the raccoon roundworm (*B. procyonis*).

Introduction

Wolverines (*Gulo gulo*) are strong carnivorous mammals belonging to the Mustelidae family. They are found in arctic, alpine, and boreal regions of the world including northern Saskatchewan. They can become infected with parasites through their broad diet.

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Polymerase chain reaction (PCR) is often used to identify infectious agents. But DNA databases and barcoding of life schemes are only as good as the data they contain, which relies on accurate species identifications. In parasitology, this often involves looking through a microscope and measuring tiny anatomical structures of worms. Once a parasite has been described by both molecular and morphological means, correct identification of the species can often be achieved by molecular means alone. This eliminates the need for wildlife (such as wolverines) to be used in terminal research (killing the animal), as the species of parasite can be identified correctly by use of molecular techniques on parasite eggs in fecal samples.

Objective The objective of this study was to identify parasitic worms in wolverines by morphological and molecular techniques.

Materials and Methods

All work was performed under University of SK animal and biosafety research ethics permits 2009-0126 and VMB-12.

To extract the parasites, wolverine intestines were thawed, cut into equal parts, incised longitudinally and placed into a sealable jar of water. The jar was then shaken and the contents were sifted through two sizes of mesh. Any visible parasites were picked out with forceps and counted under a microscope[1,2]. The filtered material was also examined for any parasites.

An experienced technician examined the heads and tails of roundworms and compared their appearance and measurements with previously published descriptions[3].

We extracted DNA from one roundworm per wolverine and amplified the COX-1 mitochondrial gene by Polymerase Chain Reaction[4,5]. PCR products were visualized using electrophoresis to determine the success of amplification.

The DNA was purified then sent for sequencing to Macrogen, Korea [6]. DNA sequences were then compared with known sequences from GenBank™ using a series of computer programs to determine relationship.

Results and Discussion

Morphological identification:

- 9 of 10 wolverines studied were infected (90% prevalence) with the nematode *Baylisascaris devosi* (Figure 1)[1].
- On average, each wolverine had 6.4 of these roundworms (range from 0 to 31).
- Length of femaleand male worms ranged from 116–215 mm and 70-106 mm, respectively, which matches that reported in the literature [3].-one *Taenia* sp. tapeworm was found in one wolverine.

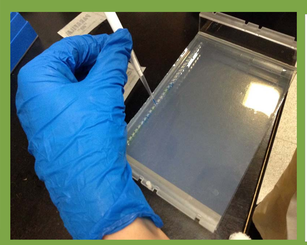
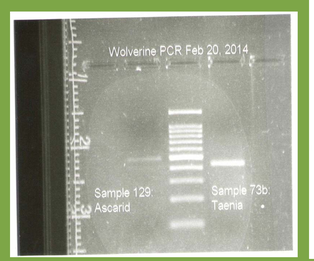


Figure 3
Baylisascaris devosi head. The three triangular denticles (small tooth-like projections) that aid the parasite to attach to the intestinal wall can be seen. This physical feature also helped in our morphological identification.



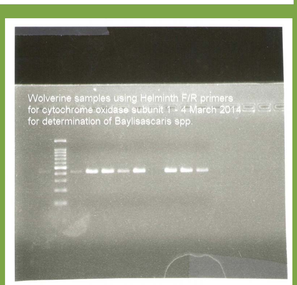


Figure 1
Gel electrophoresis results (agarose gel), showing position of a region of the COX 1 gene. Ladder represents different sizes of DNA fragments (standard). Each horizontal band next to the ladder is a COX 1 gene fragment from a single worm from different wolverines.

Molecular Identification

-Sequences of the COX1 gene were obtained for 9 *B. devosi* (identified on the basis of morphology) and 1 tapeworm (Figure 2).

-No known genetic matches were found in Genbank (Figure 2). This is because the COX 1 gene has not previously been described for *B. devosi*.

- There are 13 genetic mutations separating *B. devosi* from its closest relative- *Baylisascaris columnaris*

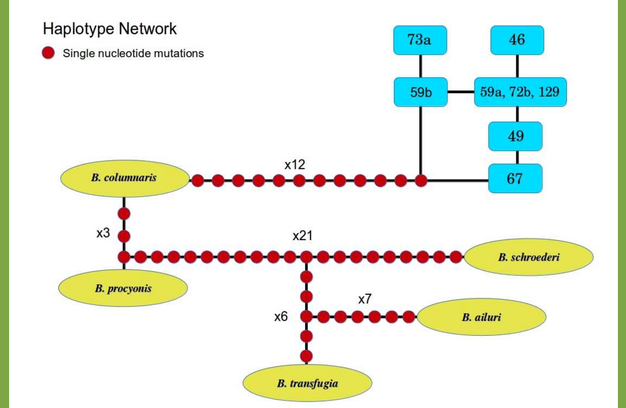


Figure 2
Haplotype network representing the relationship between our samples and known sequences from GenBank™. Each dot represents a single nucleotide mutation.

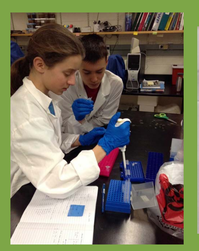
Conclusion

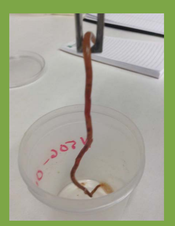
-This is the first time *Baylisascaris devosi* has been identified using molecular techniques. The resulting sequences will be entered into GenBank™

-This is important because identification by molecular means offers a quick, non-invasive molecular method to identify this parasite based on worm eggs in wolverine fecal samples, which has important implications for wildlife conservation.

- This also has implications for diagnosis of human infections. The closest known relative to *B. devosi* is *B. columnaris from skunks,* which has the potential to be infectious to humans (zoonotic) similar to its relative the raccoon roundworm (*B. procyonis*)

-Both morphological and molecular techniques are needed for accurate identification of parasites





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