Species Identification of *Dictyocaulus Found in Domesticated and Wild Ruminants*

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Abstract

Dictyocaulus, or large lungworms, are a genus of parasitic nematodes that infect cattle (Bos taurus), horses (Equus caballus), deer and other ruminants. The lungworm has been found to cause disease, specifically parasitic pneumonia, and may be severe enough to kill the host. There is some confusion in the literature whether deer and cattle are infected with the same or different species of lungworm. This is important for ranchers as to how much effort they put into keeping deer away from cattle. Molecular differences of *Dictyocaulus* species found in cattle, red deer (Cervus elaphus) and white-tailed deer (Odocoileus virginianus) were determined by amplifying the major sperm protein 1 (MSP1) gene and the mitochondrial cyclooxygenase (COX-1) gene of ribosomal DNA and then using PCR sequencing. The first round of samples sent for sequencing came back with high percent identity matches. Our results to date give samples E6 and CN5, cattle originating from Mississippi, using the Cox-1 primer identified as *Dictyocaulus* Viviparus. Also using Cox-1 primer, sample e10 (red deer) identified as *Dictyocaulus* eckerti. Using the MSP1 primer, sample W9 (white-tailed deer) identified as *Dictyocaulus* eckerti. E6 using MSP1, identified as *Dictyocaulus* Viviparus. Sample T100 (white-tailed deer) using MSP1 identified as *Dictyocaulus* eckerti. Sample T (white-tailed deer) using MSP1 identified as *Dictyocaulus* eckerti for the forward strand, and *Dictyocaulus* capreolus on the reverse stand. All identifications were as expected apart from D. capreolus. More samples will be tested with MSP1, COX-1, and ITS2 primers to determine species identity and phylogenetic relationships.

Introduction

Dictyocaulus is a genus of nematodes that are found in the lungs of many different species of mammals are known to cause parasitic bronchitis in a wide range of wild and domesticated ruminants. Infection of the parasite can also cause symptoms such as coughing, rapid shallow breathing, nasal discharge, pyrexia, increased pulse rate, weight loss and diarrhea.

Dictyocaulus has been researched due to the risk of wild ruminants, such as deer, causing infection in domesticated livestock. Other studies have determined whether *Dictyocaulus* found in different species of mammals are different species (Schnieder T, et al. 1996). By determining if there are differences in *Dictyocaulus* farmers would be able to have more options to prevent contamination from wild animals, as well as more options to treatment of the particular *Dictyocaulus* species. To date, species of *Dictyocaulus* in white-tailed deer (*Odocoileus Virginianus)* has not been analyzed (CITE). In many studies, mostly in Europe, it has been found that *Dictyocaulus* found in deer can be identified as *D.* eckerti (Höglund J, et al. 2003). *Dictyocaulus* found in cattle have been identified as D. Viviparus (C Epe, et al. 1997). This experiment was done to determine species of *Dictyocaulus* found in North American cattle *(Bos Taurus),* white-tailed deer (*Odocoileus Virginianus)* and red deer *(Cervus elaphus)* from New Zealand. Genomic DNA was amplified using PCR with three different primers (MSP-1, COX-1, ITS2)(CITATIONS AND)(TABLE 1) and sequenced to examine DNA.

Materials and Methods

- Missouri strain lungworm samples were obtained from Sara Green (Fulton, Missouri). The recovered worms came from infections caused by a six-year-old laboratory strain of larvae. Worms were stored in PBS and frozen at –20ºC.
- Two vials of Mississippi strain cattle lungworm were donated by Louisiana State University. One vial contained lungworm stored in 10% formalin collected between 1990 and 2000. The other vial contained lungworm stored in 70% ethanol recovered in May 2001.
- Regional samples of cattle lungworm were collected from a calf allowed to graze in an infected pasture in Viroqua, Wisconsin (Wisconsin strain). The calf was sacrificed after infection became patent and the lungworms were collected and stored in PBS and frozen at –20ºC.
- One vial red deer lungworms were donated by Dr. Marion Johnson, AgResearch Invermay, Mosgiel, New Zealand and stored in 70% ethanol.
- White-tailed deer lungworms were collected by the investigators from the lungs of legally harvested white-tailed deer at Frontenac State Park, Minnesota in November 2001 and White-Water State Park (year). Shortly after harvesting, the lungs were dissected using scissors starting at the trachea and cutting down through the air passageways. Recovered lungworms were stored in PBS and frozen at –20ºC.

DNA Extraction

DNA was extracted using 10% Chelex following a standard protocol. The sample was then quantified using the Nanodrop.

PCR/Gel Electrophoresis

A polymerase chain reaction was performed on each sample of lungworm DNA (Table 2). For COX-1 and ITS2 primers, initial denaturation was set at 94°C for 3 minutes. 34 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 minute, and extension for 72°C for 1 minute. A final cycle of extension at 72°C for 5 minutes was completed before the samples were held at 12°C for infinity. For samples with MSP-1 primer, initial denaturation was set at 95°C for 2 minutes. 34 cycles followed with denaturation at 95°C for 30 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 1 minute. After the completion of 34 cycles, a final extension at 72°C was held for 10 minutes, followed by 12°C for infinity. After amplification, samples were analyzed using a 1.6% gel with 3μM of SYBR (SYBR Safe DNA gel stain *10,000X Concentrate in DMSO*, by Thermo Fisher Scientific). The gel was visualized in the Analytic JENA UV illuminator and photographed.

Sequencing

PCR Samples were prepared in 50 μL volumes. Eight μL of the sample were used to visualize on the gel. If successful, PCR products were sent to Idaho State Molecular Research Core facility for sequencing.

Results

PCR products were observed for correct band size. Samples that came back with strong, consistent bands of the correct size were sent off to sequencing.

Table 1. Primer Sequences Used to Amplify *Dictycaulus* DNA

Lungworm DNA Primers	Primer	Primer Sequence
	MSP – 1 F	5' - TCG ATG AAG AAC GCA GCC AG - 3'
	MSP - 1 R	5' - TTC TAT GCT TAA ATT CAG GGG GTT GTC - 3'
	COX -1 F	5' - GCG TTA GAA ACG GAG ATT TGA - 3'
	COX – 1 R	5' - CCA CGA GAT TTA CAT TTG CC - 3'
	ITS2 F	5' - CTG CTC AAT GAT TTT TTA AAT TGC TGT - 3'
	ITS2 R	5' - GTC TGA ACT CAG ATC AAG T - 3'

Table 2. Baseline Master Mix for Amplifying *Dictyocaulus* DNA using the COX1, ITS2 and MSP1 Primers

Component	Concentration	Volume Used (ul)	Manufacturer
Green DreamTaq	2x	12.5	Thermo Scientific
$MgCl_2$	25 mM	1	Fisher BioReagents
Primer F	10μM	0.75 μL	
Primer R	10μM	0.75 μL	
DNA	~Variable (10-50ng/μL)	1.25 μL	~
Sterile Water	-	8.75 μL	Thermo Scientific

Samples sequenced were blasted using NBCI blast search to determine the species of *Dictyocaulus* for each of the primer sets. The results found sample E6 (cattle from Mississippi) using both the COX-1 and MSP-1 primers came back with D. Viviparus. Sample e10 (red deer) using COX-1 came back with D. eckerti. Continue with rest of chart and new samples. PAIGE WHAT IS THIS

Table 3. DNA Blast Results of Lungworms Isolated From Cattle, White-Tailed Deer and Red Deer Using the COX1 Gene Segments.

Sample	Primer	% identity	Genus/species	Total sequence length
E5	COX1 F	99.02	D. Viviparus	586
E5	COX1 R	98.64	D. Viviparus	335
E6	COX1 F	97.62	D. Viviparus mitochondrial DNA, complete genome	341
E6	COX1 R	98.7	D. Viviparus mitochondrial DNA, complete genome	339
e4	COX1 F	98.69	D. eckerti	481
e4	COX1 R	98.02	D. eckerti	336
e10	COX1 F	98.4	D. eckerti mitochondrion, complete genome	339
e10	COX1 R	97.85	D. eckerti mitochondrion, complete genome	338
CN1	COX1 F	99.36	D. Viviparus	337
CN1	COX1 R	99.04	D. Viviparus	336
CN5	COX1 F	98.93	D. Viviparus	658
CN5	COX1 R	99.05	D. Viviparus	336
CN6	COX1 F	99.02	D. Viviparus	337
CN6	COX1 R	98.22	D. Viviparus	335
T	COX1 F	98.05	D. Viviparus	339
T	COX1 R	97.64	D. Viviparus	336
T 1:100	COX1 F	98.73	D. Viviparus	338
T 1:100	COX1 R	98.67	D. Viviparus	334
Q	COX1 F	85.19	D. Caperolus	448
Q	COX1 R	98.98	D. Viviparus	336
R	COX1 F	97.17	D. Viviparus	645
R	COX1 R	98.05	D. Viviparus	336
F5	COX1 F	98.37	D. Viviparus	337
F5	COX1 R	97.69	D. Viviparus Bisontis Isolate	333
W1	COX1 F	88.41	D. Caperolus	440
W1	COX1 R	88.56	D. Viviparus	335
W5	COX1 F	88.81	D. Caperolus Isolate D197	453
W5	COX1 R	88.52	D. Viviparus	334
W9	COX1 F	90.58	D. Viviparus	349
W9	COX1 R	93.2	D. Vlviparus	342

Table 4. DNA Blast Results of Lungworms Isolated From Cattle, White-Tailed Deer and Red Deer Using the MSP1 Gene Segments.

Sample	Primer	% identity	Genus/species	Total sequence length
CN1	MSP1-F	92.71	D. Viviparus isolate SKa3	703
CN1	MSP1-R	87.1	D. Viviparus isolate SKa3	651
CN5	MSP1-F	97.69	D. Viviparus isolate Ska3 major sperm protein (MSP1) gene, partial cds	
CN5	MSP1-R	98.06	D. Viviparus isolate Ska3 major sperm protein (MSP1) gene, partial cds	
CN6	MSP1-F	93.67	D. Viviparus isolate SKa3	
CN6	MSP1-R	87.25	D. Viviparus isolate SKa3	924
W1	MSP1-F	89.77	D. Eckerti Isolate F10NZ	507
W1	MSP1-R	Cannot determine		288
W3	MSP1-F	86.24	D. Eckerti Isolate F10NZ	515
W3	MSP1-R			395
W5	MSP1-F	89.77	D. Eckerti Isolate F10NZ	669
W5	MSP1-R			317
W9	MSP1-F	68.1	D. eckerti isolate F8NZ major sperm protein (MSP1) gene, complete cds	353
W9	MSP1-R	78.02	D. eckerti isolate F9NZ major sperm protein (MSP1) gene, complete cds	357
E5	MSP1-F	89.67	D. Viviparus isolate SKa3	945
E5	MSP1-R	82.26	D. Viviparus isolate SKa3	930
E6	MSP1-F	99.14	D. Viviparus isolate Ska3 major sperm protein (MSP1) gene, partial cds	361
E6	MSP1-R	99.15	D. Viviparus isolate Ska3 major sperm protein (MSP1) gene, partial cds	358
T100	MSP1-F	70.49	D. eckerti isolate F8NZ major sperm protein (MSP1) gene, complete cds	357
T100	MSP1-R	81.16	D. eckerti isolate F9NZ major sperm protein (MSP) gene, complete cds	358
T	MSP1-F	70.12	D. eckerti isolate F8NZ major sperm protein (MSP1) gene, complete cds	347
T	MSP1-R	78.78	D. capreolus isolate CaAlg1 major sperm protein (MSP1) gene, partial cds	354

Discussion

DNA was extracted from *Dictyocaulus* lungworm species found in cattle from Missouri, Mississippi and Wisconsin, Red Deer from New Zealand and White-Tailed Deer from Frontenac State Park… …….

Conclusion

Bring up formalin and DNA storage

- Red Deer lungworms from New Zealand have greater than 97% to *D. Viviparus* using COX-1 gene segment.
- Cattle lungworms from Wisconsin, 88% and inconclusive……
- 2 samples of cattle lungworms from Wisconsin have greater then 88% identity to *D. Caperolus isolate*.
- Cattle lungworms from Missouri have greater than 98% identity to *D. Viviparus* using the COX1 gene segment and greater than 87% identity to *D. Viviparus isolate SKa3* using MSP1 gene segment.
- White-Tailed deer lungworms from Frontenac State park have greater than……
- 1 sample of White-tailed deer lungworms from Frontenac State park have greater than 85% identity to *D. Caperolus.*
- Cattle lungworms from Mississippi

Using this information, a phylogenetic tree can be constructed using ITS2, COX1 and MSP1 primers to demonstrate genetic relation between species of *Dictyocaulus.*

Future Research

To continue these studies, more samples of *Dictyocaulus* found in species of domesticated and wild ruminants will be amplified, sequenced and analyzed to determine whether *Dictyocaulus* found in different mammals are different species of parasite.

Literature Cited

- **D. Viviparus ID:** C Epe, G.V Samson-Himmelstjerna, T Schnieder,.Differences in a ribosomal DNA sequence of lungworm species (Nematoda: Dictyocaulidae) from fallow deer, cattle, sheep and donkeys, *Research in Veterinary Science* 62, no.1 (1997) 17-21. https://doi.org/10.1016/S0034-5288(97)90173-9.
- **EUROPE PHYLOGENY:** Höglund J, Morrison DA, Divina BP, Wilhelmsson E, Mattsson JG. Phylogeny of Dictyocaulus (lungworms) from eight species of ruminants based on analyses of ribosomal RNA data. Parasitology. 2003 Aug;127(Pt 2):179-87. https://doi.org/10.1017/S0031182003003366
- **Differences:** Schnieder, T., Epe, C. & Samson-Himmelstjerna, G.v. Species differentiation of lungworms (Dictyocaulidae) by polymerase chain reaction/restriction-fragment-length polymorphism of second internal transcribed spacers of ribosomal DNA. *Parasitol Res* **82,** (May 1996): 392-394 https://doi.org/10.1007/s004360050134
- **ITS2 Primers!!** von Samson-Himmelstjerna G, Woidtke S, Epe C, Schnieder T. Species-specific polymerase chain reaction for the differentiation of larvae from Dictyocaulus viviparus and Dictyocaulus eckerti. *Vet Parasitol* 68, no.1-2 (January 1997) 119-26. https://doi.org/10.1016/S0304-4017(96)01064-3
- **MSP**
- **COX!**

Acknowledgments

Thank you to Dr. Kimberly Bates for allowing us to participate in her ongoing research and for assisting us when needed. We would also like to thank Erika Vail for finding and providing us with the resources we used.