DNA Extraction of Lungworms in Odocoileus virginianus, Bos taurus and Cervus elaphus

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Abstract

Analysis of the genetic diversity of organisms plays an important role in research of related species and clinical applications. Lungworms (Dictyocaulus spp) have been identified in many species of ruminants. This study aimed to investigate the genomic variations of lungworms species, which could potentially lead to practical control methods and therapeutics in the distinct species of lungworms. To determine genetic relatedness, lungworm DNA was isolated from white-tailed deer (Odocoileus virginianus), Louisiana cattle (Bos taurus), Mississippi cattle, New Zealand Red deer (Cervus elaphus) and Wisconsin cattle and extracted, PCR amplified and visualized using agarose gel electrophoresis of the amplified PCR products. Currently, the PCR protocol has been unreliable and protocols are being designed to give more consistent results. Once the PCR is fully functional, the next steps will be DNA cloning and sequencing for further investigation of the different species.

Key words: Lungworms, PCR, Genomic diversity, Agarose gel, DNA Cloning, DNA Sequencing, electrophoresis.

Odocoileus virginianus | Bos taurus | Cervus elaphus

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by several parasitic nematodes such as *Dictyocaulus viviparus*in cattle (*Bos taurus*). Different species of *Dictyocaulus*infect other ruminants such as *D. eckerti*in fallow deer (*Dama dama*), *D. filaria*in sheep (*Ovis aries*) and *D. arnfieldi*in donkeys (*Equus africanus asinus*) (Epe et al. 1997). These nematodes settle in the lungs and trachea, causing severe respiratory problems. *D. viviparus*is one of the most common in cattle and other ruminants and this greatly affects agricultural production, income, and food supply. *D. viviparus* lives in the bronchi and bronchioles of the lungs and are responsible for parasitic bronchitis in cattle. The lungworm life cycle begins by ingesting infectious larvae through food, pasture, etc (figure 2). The infective larvae then penetrate the intestinal wall and enter the lungs via the bloodstream. They develop into adult and larvae are passed in feces and ingested on pasture by other ruminants. In the lungs, they irritate the airways of these animals, causing shortness of breath, fever, cough and weight loss. Temperate regions or areas with wider temperature ranges throughout the year are mostly affected (Anderson 1981) and as the temperature rises due to climate change, these parasites will likely migrate to more temperate regions. Parasites are one of the causes of mortality in White-tailed deer (*Odocoileus virginianus*). They obtain larvae while feeding on vegetation (Mason, 1985), which makes it necessary to monitor and maintain healthy deer. In New Zealand *Dictyocaulus eckerti*infects red deer and is the major cause of mortalities in young farmed red deer during autumn and winter.

It is believed that deer species and cattle share lungworm infections by sharing infected pasture. However, many lungworms found in deer species in Europe have been reclassified to *D. eckerti*(Divina et. al, 2002) and to date no one has looked at the lungworms found in white-tailed deer. A cross-transmission study between deer fawns and larvae isolated from cattle indicate that cattle and white-tailed deer may be harboring different species (Bates et. al, 2000). This study will use the ITS2 gene of rRNA to determine genetic relatedness between *Dictyocaulus*isolated from cattle, white-tailed deer and New Zealand red deer.

Figure 1. Lungworm view in Microscopy

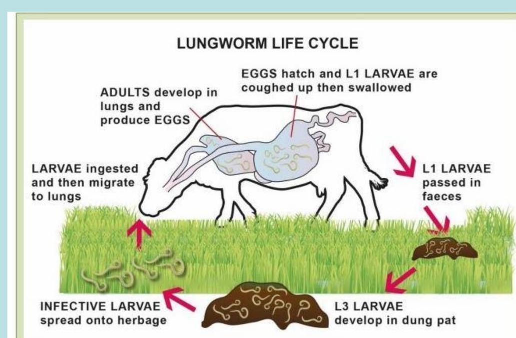

Figure 2. Lungworm life cycle in cattle and deers

Methods

DNA was extracted from individual *Dictyocaulus* isolated from cattle from Wisconsin, cattle in Mississippi, white-tailed deer from Minnesota and red deer from New Zealand using three different protocols. The first protocol was the Chelex protocol which required a chelex solution to be made consisting of 1.00 g of Chelex with 10 mL of autoclaved water. 200 uL of the Chelex solution was pipetted into a PCR tube and a single lungworm was added to the same tube, which was then incubated for 5 minutes at 100 degrees Celsius. After incubation, the solution was centrifuged, and the supernatant was collected to be quantified. This procedure was performed once again for a different lungworm; however, Proteinase K was added to the mixture.

The second protocol was the DNeasy Blood and Tissues Protocol. For this protocol, the lungworm was cut up into fragments and placed into Buffer ATL and then proteinase K was also added. This mixture was then incubated at 56 degrees Celsius on a rocking platform for 3 hours. After incubation, Buffer AL and ethanol were added. Centrifugations were performed with further addition of Buffers AW1 and AW2 and then finally eluted twice with Buffer AE. The supernatant from the last round of centrifugation was collected and quantified.

The third protocol first required the lungworm to be heat-dried and then inserted into a tube consisting of TE buffer, 0.5% SDS, and proteinase K. It was then incubated at 37 degrees Celsius for 1.5 hours in a shaking incubator. After digestion, tubes were spun down in a microcentrifuge and the supernatant was transferred to a new tube which contained isopropanol. Further centrifugation steps were required with the supernatant being collected at each step. After the final centrifugation, the pellet was collected, put into a dry bath, and was then dissolved in water at room temperature. This final solution of the dissolved pellet in water was then quantified.

The concentration of each isolate was determined using nanodrop machine to ensure that the DNA concentration was within 10 ng/ul - 50 ng/ul. Polymerase Chain Reaction (PCR) was performed on each sample using a mastermix (Table 1). There are three main steps in PCR, which are denaturation, primer annealing and finally extension. Denaturation takes place to denature and separate the Template DNA by heating the solution. Annealing of the primers is done by reducing the temperature to allow the primers to bind to the correct parts of the template DNA. The final stage, extension, allows the enzyme DNA Taq Polymerase to bind the correct nucleotides to the correct base pair on the DNA (Table 2). The amplified PCR products were visualized on 1.5% agarose gel after gel electrophoresis.

Table 1: PCR Master Mix Composition

Component	Concentration	Volume per Individual reaction (uL)
$MgCl_2$	1 mM	1
Forward Primer	10 uM	1
Reverse Primer	10 uM	1
Dream Taq	2x	12.5
Sterile Water	--	9.5
DNA	1-50 ng/ul	1

Table 2: Thermal Cycling Conditions

Step	Temperature(°C)	Time	Number of Cycles
Initial denaturation	94	2 minutes	1
Denaturation	94	30 seconds	30-35
Annealing	64	30 seconds	
Extension	72	1 minute	
Prolongation	72	5 minutes	1

Results

At the beginning of fall semester, successful amplification of white-tailed deer and Wisconsin cattle were achieved, but were not consistent. On the other hand, Red deer did not amplify successfully (Fig. 3).

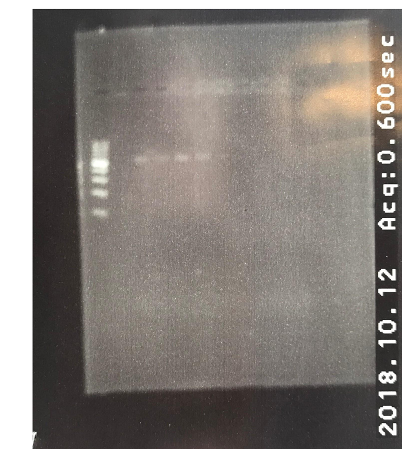

Fig. 3: Red deer samples. No results.

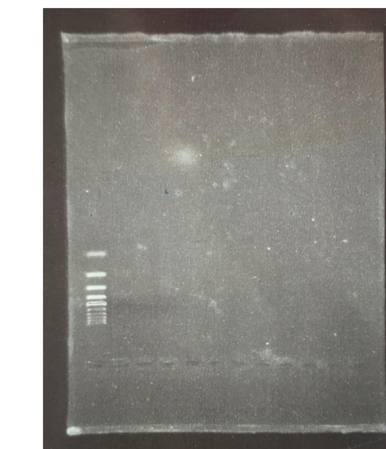

Fig. 4: White-tailed deer, Lane 1: Ladder, Lane 2: P_a, Lane 3: P_b, Lane 4: P_c, Lane 5: P_d, Lane 6: P_e.

A PCR was run on all DNA in order to check the quality of DNA. The primer, Cytochrome c oxidase, was included in the master mix. The negative control showed up on the gel, indicating contamination. The source of contamination was unknown.

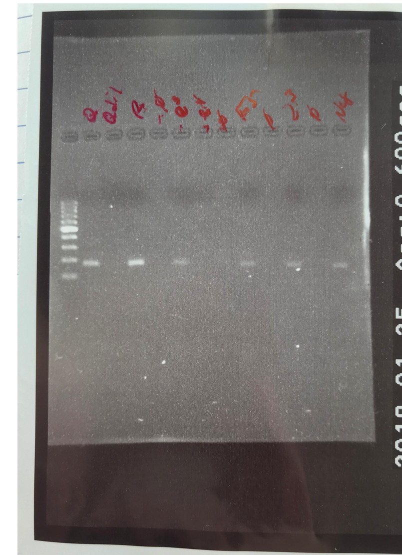

Fig. 3: Contamination of negative control. Lane 1: Ladder, Lane 2: White-tailed deer, Lane 3: White-tailed deer (1:100 dilution), Lane 4: White-tailed deer, Lane 5: N/A, Lane 6: New Zealand Red Deer (1:10 dilution), Lane 7: New Zealand Red Deer (DNA did not freeze), Lane 8: N/A, Lane 9: Louisiana cattle in Formaline, Lane 10: N/A, Lane 11: Wisconsin Cattle, Lane 12: N/A, Lane 13: negative control.

Four different protocols were used to extract fresh lungworm DNA from cattle which gave a positive result. There was no contamination of the negative control so the PCR was successful.

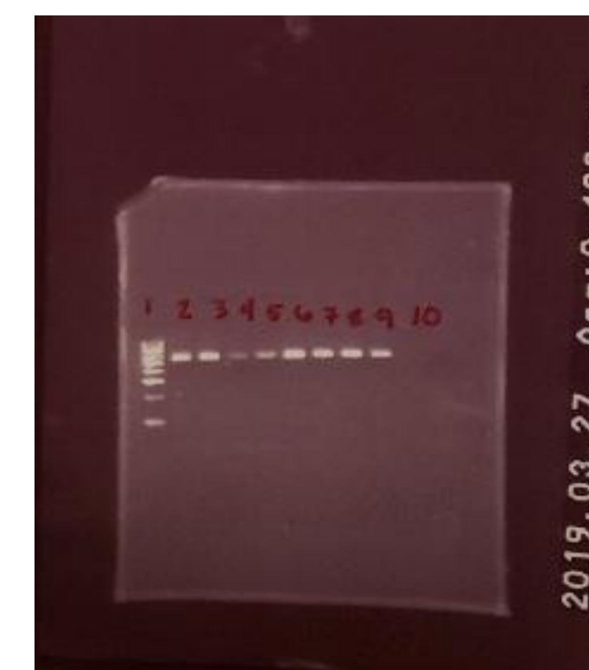

Figure 4: ITS2 rRNA amplification in Dictyocaulus using 4 different DNA extraction methods. Bands for each sample were successfully visualized after performing gel electrophoresis. The band shown on each lane are as follows: Lane 1: Ladder, Lane 2: Diluted chelex with proteinase K, Lane 3: Chelex without Proteinase K, Lane 4: Protocol 3, DNA 1, Lane 5: Protocol 3, DNA 2, Lane 6: Elusion 1 worm 1, Lane 7: Elusion 1 worm 2, Lane 8: Elusion 2 worm 1, Lane 8: Elusion 2 worm 2.

Discussion

The purpose of this research study was to determine the gene from lung-worms that infect cattle from Wisconsin, cattle from Mississippi, white-tailed deer from Minnesota and red deer from New Zealand, and to then sequence them to determine if there are any similarities. When first attempting to amplify the DNA of lungworms from cattle and deer, there were inconsistent results. In order to determine what went wrong, it was decided that the focus was first going to be on the cattle lungworm DNA, not the white-tailed deer. The first step taken to solve this issue was to increase the concentration of the agarose gel, however, this proved to have no effect on the amplification of DNA. Because the gel was not the issue, the next step was to use a new primer, Cytochrome c oxidase, which would check for the viability of the DNA. The use of this new primer allowed for adequate amplification of the DNA, however, the negative control showed up as well, indicating that contamination had occurred. Because of this, the results could not be used and it was decided that new lungworm DNA would be extracted using the different protocols mentioned in the methods section. In addition to fresh DNA, all new reagents were used. This allowed for a completely fresh start to make sure there would be no contamination. This new DNA was then amplified and run on a gel and produced favorable results for all four DNA extraction protocols.

Future Research

In the future, the DNA amplification process will be continued using the cattle DNA that was extracted using the 4 different protocols and then cloned using bacterial plasmids for replication. Once successful replication has been achieved, the DNA will be sent in to be sequenced which can then be compared to published resources. Also, new lungworms from white-tailed deer will be obtained and the same process will be repeated. From there, it will be concluded whether or not the lungworms infecting the deer and cattle are of the same species.

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Acknowledgments

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