Molecular Identification of Cestodes from Mallards and Canvasbacks around the Winona MN Area

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Abstract

Parasites can have a significant effect on an ecosystem if they become too abundant due to the multitude of animals that play a role in their life cycle. This research is a part of a larger class project for BIO 462 identifying parasites that are commonly found in or on waterfowl from around the Winona, Minnesota area. Ducks were donated from local hunters and dissected to collect their endo and ectoparasites. Parasite specimens were stained and mounted to look for morphological characteristics that assisted in identifying the parasites using light microscopy. DNA was extracted from individual cestodes, and gel electrophoresis and PCR were used to amplify select sections of the genome. The DNA was sequenced and compared to DNA samples in GenBank to determine if the samples were cestodes or if a new species was found. Results from GenBank are pending currently. More specimens will be processed to obtain additional results and proper identification. These findings could help identify not only what parasites are common in certain species of ducks, but also provide additional information on the abundance of parasites within waterfowl in our local area.

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Introduction

Parasitic infections worldwide amongst humans and animals are commonly caused by helminths. Small game animals such as ducks can be a model for the prevalence of helminths in our local ecosystem. Helminths include nematodes (roundworms), trematodes (flatworms), and cestodes (tapeworms). Ducks and other types of waterfowl host a variety of parasites, both helminth and otherwise. These parasitic infections affecting a population have been shown to lead to decreases in duck population with the possible outcome of mass death (Amundon et al., 2016). Cestodes are segmented worms that are parasitic and specialized to many different species of animals including waterfowl. The way cestodes spread is primarily in water (Henneman, 1996) making ducks vulnerable due to infection by contaminated water. Cestodes are primarily found in the gastrointestinal tract (G.I. tract) of ducks and vary greatly in size and shape. While some of the tapeworms may be differentiated morphologically, DNA sequencing is the best method to differentiate parasite species.

Ducks for our Spring 2022 experiment were provided by local hunter donations. The species of ducks that yielded cestodes for viable DNA extraction included mallards *(Anas platyrhynchos)*, ring-necked *(Aythya collaris)*, canvasback *(Aythya valisineria)*, and wood ducks (*Aix sponsa*). One of the most common ducks in the United States and most prevalent in our region is the mallard. Mallards are dabbling ducks, who feed on the surface of the water and may feed off land. This is opposed to diving ducks such as the canvasback, which dive to feed from below the water's surface. Dissection of the ducks collected allowed for retrieval of all parasites infecting the duck, such as helminths of the GI tract or lice in their feathers. Since these ducks are feeding in different niches of the ecosystem they were exposed to different parasites, with cestodes being the focus of our study.

The cestodes found in duck dissections can be identified by examining the morphology of the worm under a microscope or by matching the DNA sequence of the cestode to a genome database. Morphological identification is notoriously difficult, with many distinctive features only being visible during developmental stages of the cestode or by observing the egg stage (van Steenkiste et al., 2014). The latter method of DNA sequence matching is more precise, which was completed by extracting DNA from parasites that were removed from the ducks and were amplified using a polymerase chain reaction (PCR). Successfully amplified samples were sent for sequencing. Waterfowl cestodes are commonly not sequenced, thus having little to no matches on databases such as NCBI's GenBank, due to research prioritizing the study of cestodes affecting humans. In addition, the cestode genome is difficult to isolate and replicate using polymerase chain reaction (PCR) tests. Promising primers were proposed in the study conducted by van Steenkiste et al., in which they created Dice1F, Dice 11R, and Dice 14R primers that were successful in amplifying extracted cestode DNA sequences for PCR analysis (van Steenkiste et al., 2014). The goal of this study was to identify the species of cestodes collected from duck dissections by using DNA sequencing and matching to a database such as GenBank.

Materials and Methods

Endoparasite Collection:

All the parasites collected were retrieved from ducks dissected during Dr. Bates' BIOL 462 course during Spring 2022 and from previous research conducted by Dr Bates' and various capstone students. Ducks were donated by local hunters and preserved by freezing over the past year. Ducks were dissected according to the protocol provided by Orlofske (2020). Parasites recovered were labeled and preserved in 80% ethanol. Representative cestodes were stained and mounted according to the protocol provided by Tkach and Orlofske (2020).

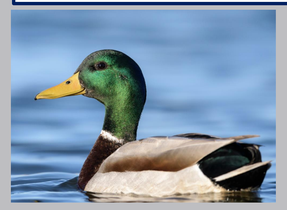
DNA Extraction:

Chelex DNA extraction was performed using 200 µl of 10% Chelex bead solution that was placed in sterile PCR tubes. Cestode samples were removed from 70% ETOH solution and allowed to air dry and then placed in the Chelex solution. The cestode samples were then crushed and vortexed for 15 seconds to allow them to mix with the solution and placed in the thermal cycler at 100 degrees Celsius for five minutes. The solution was then centrifuged for five minutes, and the supernatant was recovered and quantified before freezing.

PCR:

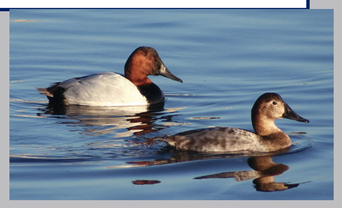
A 25µl reaction was prepared for the PCR. PCR was used to amplify a known portion of cestode mitochondrial DNA known to be 700 and 900 bp respectively. A master mix was created containing the following: 12.5µl Dream Taq PCR Master Mix, 1µl Dice 1F forward primer and 1µl of Dice 11R and 1µl Dice 14R reverse primers, 3.5µl magnesium chloride, and 6µl sterile water (van Steenkiste et al., 2014). The master mix was then combined with 3 µl of water to create the negative control and 1µl (between 10-50 ng) of cestode DNA was used.

A 314 bp region amplifying the 12s rDNA gene was performed by creating a master mix containing the following: 12.5 µl Dream Taq, 1µl 12 sF forward primer and 1µl 12 sR reverse primer, 1µl magnesium chloride (von Nickish-Rosengegk et al., 1999). The master mix was combined with a variable amount of water to create the negative control and variable amounts of cestode DNA was added depending on the concentration of DNA that was extracted. The added DNA concentration was measured in ng/µl and a minimum of 100ng-800ng of extracted DNA was added to each of the samples. Amplified products were visualized on a 1.6% agarose gel. Successful products were sent off to Idaho State Molecular Research Core Facility for sequencing.



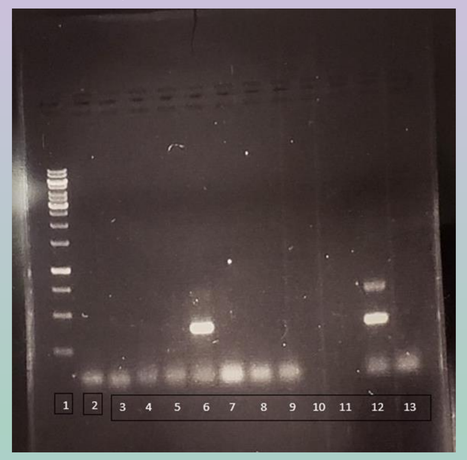
Left: Mallard Duck

Right: Male and Female Canvasback Duck



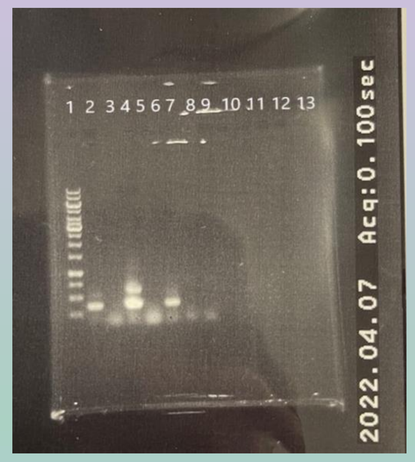
Results

Figure 1: Gel Electrophoresis of PCR products from Duck #55 1R, 14R, and 12S Primers using the DICE 1 F'/11 R', DICE 1 F'/14R" and 12sF' and R' primers.



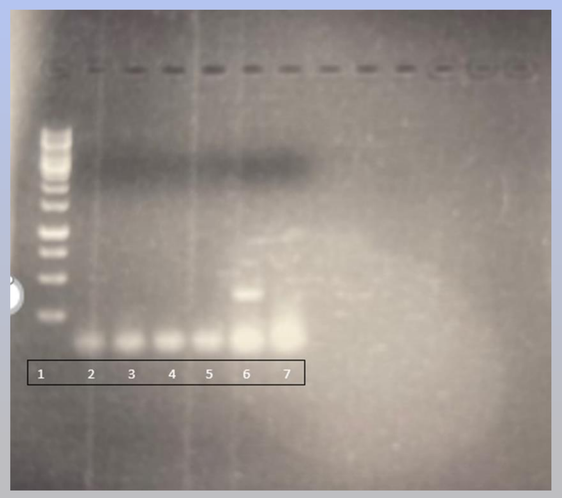
The DNA sample collected from the GI of duck #55 (canvasback) was amplified using PCR and its base pair size was determined using gel electrophoresis. Well 1 contained the ladder, well 2 contained the control with 14R and water. Well 3 contains 14R and DNA, well 4 contains 11R and DNA, well 5 contained 11R and water, well 6 contains 12S and DNA and well 7 contains 12S and water. Well 8 contains 14R and water, well 9 contained 14R and DNA. Well 10 contained 11R with DNA and well 11 contained 11R with water. Well 12 contained 12S with DNA and well 13 contained 12S with water. Well 6 showed approximately 300- 350 bp. This was replicated for each student to fill a total of 13 wells of the gel. The results yielded prominent bands between 250 bp and 500 bp in wells 6 and 12. A double band was observed for one student's sample, with the second band measuring at approximately 600 bp shown in well number 12 which contained 12S DNA (Figure 1).

Figure 2: Gel Electrophoresis of PCR products from Duck #63 1R, 14R, and 12S Primers using the DICE 1 F'/11 R', DICE 1 F'/14R" and 12sF' and R' primers.



The DNA sample collected from the GI tract of duck #63 (canvasback) was amplified using PCR and its base pair size was determined using gel electrophoresis. Well 1 contained the ladder, wells 2, 4, and 6 contained 12S samples containing cestode DNA, with controls containing water in wells 3, 5, 7, and 8. Each 12S DNA sample formed a prominent band between 250 bp and 500 bp at as estimated 300 bp. Well 4 contained 12S DNA samples that resulted in a double band, with the second band forming at approximately 600 bp (Figure 2).

Figure 3: Gel Electrophoresis of PCR products from Duck #55 (Mallard) DNA Samples 1R, 14R, and 12S Primers using the DICE 1 F'/11 R', DICE 1 F'/14R" and 12sF' and R' primers.



The DNA sample collected from the GI tract of duck #55 (mallard) was amplified using PCR and its base pair size was determined using gel electrophoresis. Well 1 contained the ladder, wells 2 and 3 contained the 11R sample and its control, wells 4 and 5 contained the 14R sample and its control, and wells 6 contained 12S with DNA and well 7 contained the 12S with water. A band formed in well 6 which contained the 12S sample with DNA measuring between 250 bp and 500 bp, approximately 350 bp (Figure 3). .

Discussion

This experiment was conducted as a class to identify and quantify the parasites inhabiting local duck populations. Cestodes were of interest due to the large amount of cestodes collected from the dissected ducks and the inability to easily distinguish morphological differences between adult cestodes for species identification. Thus, we completed genetic identification of the cestodes collected by DNA sequencing. DNA was extracted from cestode segments and amplified using PCR with primers provided by the study conducted by van Steenkiste (2014). These primers included the Dice 1 forward primer in mixture with either 11 reverse or 14 reverse primers. For cestodes specifically, the 12S forward and reverse primer were also used.

DNA extraction was performed, in which 16 samples were successfully extracted. After PCR amplification and gel electrophoresis, 11 of the 16 total samples yielded results and were sent out for genome sequencing. In testing the 11 samples of extracted DNA, many of the samples tested using PCR yielded the same results, approximately 300-350 base pairs. This correlated to the expected 314 base pair size that was extracted in the study conducted by von Nickish-Rosengegk (1999) for the 12s primers. This result shows that the DNA extracted was from the cestodes collected, however, it does not identify the specific species of cestode present.

Additional assays were performed to determine the genome sequence of the DNA extracted from the 11 samples. The genome sequences are to be cross checked with GenBank to determine what specific species of cestode were found within the ducks dissected. GenBank does not contain all genomes for every species. The genome information that was cross referenced with the online database was from ducks rather than from human infections, which makes up much of the online database gene sequencing. This could result in incomplete findings due to the absence of a DNA sequence to cross reference in GenBank. Results from GenBank are currently pending and will be updated when they are received. Further studies of local waterfowl could be performed to add information to the current database so proper identification could be determined in the future.

Conclusions

Many of the ducks dissected had numerous parasites, including helminths and other parasites such as lice. This study was primarily focused on the cestode population. Cestode DNA was successfully extracted and amplified using PCR in 11 samples. Gel electrophoresis of the samples yielded results close to the target of 314 bp for many samples, which identifies the DNA as belonging to a cestode but does not identify the specific species (von Nickish-Rosengegk et al., 1999). Some additional samples that amplified in the 700 and 900 bp range, corresponding to the DICE1/11R and DICE1/14R primers, respectively, were also sent for sequencing. DNA from those samples was sent to GenBank for crossmatching analysis and confirmatory genome identification. Currently identification is pending due to results not being received. Further studies should be performed to further grow GenBank database as this would assist in proper identification for future studies.

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