

# Investigation of mourning doves (Zenaida macroura) as sentinels of environmental contamination with Neospora caninum



## Abstract

Infection with *Neospora caninum* is a major problem in cattle that causes spontaneous abortions and associated economic losses. We aimed to determine if mourning doves (Zenaida macroura) could serve as intermediate hosts for this protozoan parasite or as sentinels for environmental contamination of infectious oocysts. We harvested brain tissue from 47 hunter-killed doves from the Forks of the River Wildlife Management Area in Knoxville, TN. in September of 2022. Tissue was stored at -20°C until molecular analyses were performed. DNA was extracted with the DNeasy Blood & amp; Tissue kit. We used the NP1 and NP2 Neospora-specific primers to detect Neospora DNA using PCR. PCR was analyzed using gel electrophoresis. We have found 0% (0/6) PCR positive samples in the subset of samples we have processed. We plan to continue this process with the remaining samples. Any positive bands will be sequenced to confirm Neospora species. If we find Neospora we will confirm that infectious oocysts are present in the Knoxville area environment and support the idea that birds may serve as intermediate hosts.

## Introduction

*Neospora caninum* is a protozoan parasite that infects livestock and companion animals. It has a heteroxenous life cycle. Intermediate hosts include cattle and deer whereas canids are the only known definitive host. *Neospora caninum* is transmitted when a canid passes the oocysts in fecal matter and it is then ingested by an intermediate host. The canid definitive host completes the cycle when ingesting infective intermediate hosts. In addition to infection by ingestion, it can also be passed from mother to offspring through the placenta. The presence of *N. caninum* is a concern for cattle that causes spontaneous abortions and associated economic losses. (De Barros et al. 2018)

Diagnosis of *N. caninum* in birds is challenging, because no test is validated. Birds can be infected with N. caninum through ingesting oocysts from the ground or in contaminated water. Little is known about the intermediate host or environmental sentinel potential impact of N. caninum in birds. Mourning Doves are the most hunted game bird species in the US (Geissler et al. 1987).



# Dr. Kathryn Purple, Kaitlyn M. Staples-Brett

#### **Materials and Methods**

We harvested brain tissue from 47 hunter-killed mourning doves (Zenaida macroura) from the Forks of the River Wildlife Management Area in Knoxville, TN. in September of 2022 (Fig. 1). Brain tissue was collected from each bird and stored at -20 degrees Celsius. DNA was extracted using DNeasy Blood & Tissue kit according to the manufacturer's instructions. We will perform PCR using Neospora specific primers NP1 (5' TAC TAC TTC TCG TGA GTT G 3') and NP2 (5' TCT CTT CCC TCA AAC GCT 3') as soon as a positive control is obtained. Negative controls, consisting of nuclease-free water, were used for PCR. PCR products will be analyzed on 1.5% agarose gel and positive samples will be sequenced for confirmation of N. caninum.



We expect to document N. caninum DNA in a portion of mourning dove brain samples. No one has looked for DNA in frozen tissues from animals in East Tennessee. We plan to continue this process with the remaining samples. Any positive bands will be sequenced to confirm Neospora species. If we find Neospora we will confirm that oocysts are present in the Knoxville area environment and support the idea that birds may serve as intermediate hosts.

If birds can serve as intermediate hosts or environmental sentinels, this could be helpful in identifying areas with a high burden of Neospora caninum oocysts in the environment. Because N. caninum infections occur in organs other than the brain, examining only the brain tissue from doves might have some limitations; however, other tissues or biological samples from the birds in this study were not available. Future studies can be done with identifying N. caninum in other organs of birds.





#### **Expected Results**

#### **Discussion**

#### **References**

De Barros, Luiz Daniel, et al. "Neospora Caninum in Birds: A Review." *Parasitology International*, vol. 67, no. 4, 2018, pp. 397–402., https://doi.org/10.1016/j.parint.2018.03.009.

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