Avian haemosporidian infection and cloacal bacterial diversity in Maine waterfowl

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1) Microorganisms – tiny organisms living in all kinds of environments

2) Microbiota – a community of microorganisms in a specific environment

3) Microbiome – a community of microorganisms & their role within a specific environment, considering environmental conditions & interactions with each other
Microorganisms – tiny organisms living in all kinds of environments

Microbiota – a community of microorganisms in a specific environment

Microbiome – a community of microorganisms & their role within a specific environment, considering environmental conditions & interactions with each other
Dysbiosis – imbalance in the natural microbiome, mostly in the gut
Dysbiosis

Dysbiosis often associated with Disease
Avian Gut Microbiome and Pathogens

Salmonella present and absent barn swallows (*Hirundo rustica*) have different cloacal microbiome diversity.

Overview – Why Birds
Why Birds?

Overview – Migration
Main Question
What is the relationship between the avian cloacal microbiome and avian haemosporidian infection in waterfowl?
Overview – Study System

Study System

Region A: Lake Christina and Lake Josephine

Region B: Unity Pond, Carlton Pond, Plymouth Pond, Madawaska Marsh, Mulligan Stream

Wood Duck (Aix sponsa)

Green-winged Teal (Anas carolinensis)

Mallard Duck (Anas platyrhynchos)

American Black Duck (Anas rubripes)
Objectives

1) Determine the prevalence, diversity, and intensity of haemosporidians in waterfowl species found in Maine.

2) Evaluate the use of qPCR for parasite detection and quantification compared to traditional microscopy in this system.

3) Examine relationships between avian haemosporidian infections and the microbiome.
Avian Haemosporidians
a.k.a. Avian Blood Parasites

Haemoproteus

Plasmodium

Leucocytozoon
Infections not always pathogenic, often chronic

*Plasmodium* spp. associated with population declines (e.g., Hawaiian honeycreepers)
Overview
Objectives 1 & 2 - Background

Ann Bryant, Olivia Choi
• Higher detection probability
• Measure parasite load ($C_t$ value)
**C<sub>t</sub> Value**

- **C<sub>t</sub> value (cycle threshold)**
- Number of cycles needed for signal to cross the threshold (background level)
- Higher C<sub>t</sub> -> lower target DNA, lower C<sub>t</sub> -> higher target DNA
Intensity (parasite load) – number of parasites within a single infected host

$qPCR\ (C_t)\ values\ correlate\ with\ parasite\ intensity\ from\ microscopy$
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Two samples

1) Blood smear  2) DNA Extraction

Analyses

Pathogen detection: qPCR and microscopy

Nested PCR: Species Identification

Overview  Objectives 1 & 2 – Methods
Prevalence varies by host species

- Wood duck (76) 62%
Prevalence varies by host species

- Wood duck (76): 62%
- Green-winged teal (17): 53%
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- Mallard duck (235): 35%

Overview
Objectives 1 & 2 – Results

Preliminary Data
Prevalence varies by host species

- Wood duck (76): 62%
- Green-winged teal (17): 53%
- Mallard duck (235): 35%
- Black duck (30): 47%
Prevalence varies by host species

(\textit{chi-square, }p = 0.012)
qPCR vs Microscopy

McNemar’s Exact $p = 0.25$

Subset of 37 paired samples
C<sub>t</sub> Correlates with parasite load

Serial dilution using synthetic positive control
Conclusions & Future Directions

• Prevalence varies across host species, and we expect parasite diversity and intensity to follow this pattern.

• Linear models: infection status and intensity with host ecological factors (sex, age, site, mass, wing length, etc.)

• Parasite infection status and microbial community diversity?
Objectives

1) Determine the prevalence, diversity, and intensity of haemosporidians in waterfowl species found in Maine.

2) Evaluate the use of qPCR for parasite detection and quantification in comparison to traditional microscopy.

3) Examine relationships between avian haemosporidian infections and the microbiome.
Salmonella present and absent barn swallows (*Hirundo rustica*) have different cloacal microbiome diversity.
Cloacal microbiome diversity and abundance can differ between avian influenza virus infected and uninfected waterfowl.
Microbiome Workflow

Extraction – cloacal swabs

PCR – V4 and V5 regions of 16S rRNA gene

Overview       Objectives 1 & 2       Objective 3 – Methods
**Terminology**

Alpha diversity – diversity within a sample

Community A

Community B

Diversity Community A > Community B
**Terminology**

Beta diversity – similarity between communities/samples

- **Community A**
  - 1/4 species shared

- **Community B**
  - 3/4 species shared

- **Community C**
  - 2/4 species shared

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Overview

Objectives 1 & 2

Objective 3 – Results
Infected birds have decreased alpha diversity compared to uninfected birds.

Subset of 30 birds using rarefied data
Beta diversity differed between infected and uninfected birds

Subset of 30 birds using rarefied data
Implications

Potential improved avian haemosporidian detection via qPCR

More efficient approach for evaluating parasite load

Relationship between infection and community diversity
   Avian blood parasite infection <-> immune system <-> microbiome
   Avian microbiome as indicator of animal health

Future direction: incorporate co-infection, immune status
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Overview  Objectives 1 & 2  Objective 3  Acknowledgements
QUESTIONS?

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