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Out with the Old and in with the New: A Comparison Between Molecular and Traditional Techniques to Identify Parasitized Birds

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Abstract

Traditionally, the identification of blood parasites has been based on visual examination of blood smears. This approach depends on individual expertise in making blood smears and identifying parasites, which can vary widely from person to person. Recent work has shown that reading blood smears is significantly less sensitive than using molecular studies in identification. Thus, the accuracy of the data can fluctuate greatly. This project compares the ability of investigators to identify infected birds using microscopes and blood smears with their ability to identify infected birds through molecular analysis of blood from the same sample.

During fall migration (September – October) 2011, raptors were trapped at the Idaho Bird Observatory near Boise, Idaho. Blood samples were collected from both jugular and wing veins. Blood smears were made and some of the blood was stored for subsequent molecular analysis. Species of interest were American kestrels (*Falco sparverius*), Cooper's hawks (*Accipter cooperi*), northern goshawks (*A. gentilis*), sharp-shinned hawks (*A. striatus),* and red-tailed hawks (*Buteo jamaicensis*).

Avian malaria parasite lineages (*Plasmodium* and *Haemoproteus* spp.) and *Leucocytozoon* occur in Accipitridae and Falconidae and were analyzed for prevalence via blood smears, DNA extractions, and Polymerase Chain Reaction (PCR) screening. By comparing and contrasting these two techniques I identify parasite prevalence that was previously undefined or misidentified through visual screening. Using molecular methods could impact the current taxonomic assessments based on parasite morphological descriptions and potentially impact future management and conservation efforts of wild raptors and their vectors.

Keywords

infected-raptor, DNA, microscopy, pcr-analysis, parasitic-prevalence, avian-malaria

Disciplines

Poultry or Avian Science

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Introduction

Traditionally, the identification of blood parasites has been based on the visual examination of blood smears. This approach depends on individual expertise in making blood smears and identifying parasites which can vary widely from person to person. Recent work shows that reading blood smears is significantly less sensitive than using molecular studies in identification. Thus, the accuracy of data can fluctuate greatly. This project compares the ability of investigators to identify infected birds using both blood smears and microscopes with their ability to identify infected birds through molecular analysis of blood from the same sample.

Methods

During the Fall 2011 migration (September – October), raptors were captured at the Idaho Bird Observatory in Boise, ID.

- Blood was drawn from either the jugular or wing vein
- Part of the blood was used to make blood smears and remainder was stored for further molecular studies
- Blood smears were later stained using *Giemsa-Write Hematological Stain*
- Slides were examined for parasites
- DNA was extracted from the stored blood using an *EZ BioResearch* mini-kit
- DNA was screened for Avian Malaria using specific *Plasmodium, Haemoproteus* and *Leucocytozoon* primers: HaemNFI, R3, F, R2, FL, and R2L


Cooper's Hawk (*Accipiter cooperi*)


Northern Goshawk (*Accipiter gentilis*)


Sharp-Shinned Hawk (*Accipiter striatus*)


Red-Tailed Hawk (*Buteo jamaicensis*)

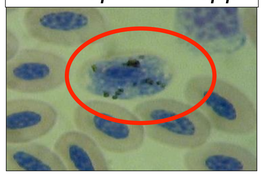

American Kestrel (*Falco sparverius*)

Traditional Approach

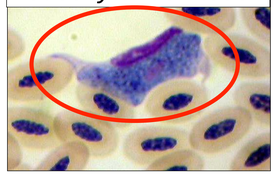
- Blood smears were analyzed through the use of a light microscope at 1,000 times magnification
- Morphological characteristics were used to verify parasitic organisms

Plasmodium relictum

Haemoproteus spp.



Leucocytozoon toddi



Presence of Parasites in Blood Samples

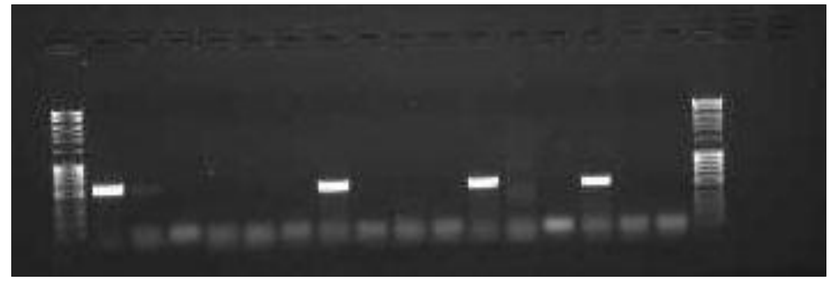
ID	Microscopy	PCR
02062	✓	✓
07766		✓
07805		✓
09147		✓
09194		
15930		
16648		✓
16682	✓	✓
16695		✓
28688	✓	✓

Results

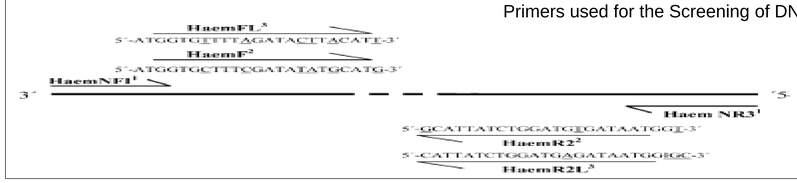
- Species within the *Plasmodium, Haemoproteus,* and *Leucocytozoon* genera were found to be parasitic
- Through Microscopy, three of ten samples were deemed parasitized
- Through PCR analysis, eight of ten samples were deemed parasitized
- The traditional method incorrectly passed five of eight parasitized samples as being free from parasites
- Only 37.5% of parasitized samples were read correctly

Molecular Approach

- Extracted DNA was analyzed using Polymerase Chain Reaction (PCR)
- Extracted DNA with PCR reagents were put through a thermal cycler to amplify Avian Malaria DNA sequence
- Amplified DNA was analyzed through electrophoresis and bands were examined under a UV light
- The presence of bands in the examination of the gel determines if parasitic DNA was extracted



Primers used for the Screening of DNA



Discussion

This comparative study reinforces the notion that visually inspecting blood smears is less accurate than confirming the presence of Avian Malaria through DNA analysis. This is due to the traditional method's limitations which "fail to register many malaria parasite infections that are picked up by PCR Screening" (Fallon & Ricklefs). By placing a preference towards the more accurate molecular approach, the misdiagnosis and improper treatment of a parasitized bird will be kept at a minimum. Further work can improve the management and conservation efforts of wild raptors and their vectors.

References

Fallon, Sylvia M., and Robert E. Ricklefs. 2008. "Parasitemia in PCR-detected Plasmodium and Haemoproteus infections in birds". *Journal of Avian Biology.* 39 (5): 514-522. Hellgren O, J Waldenström, and S Bensch. 2004. *Figure 1* "A new PCR assay for simultaneous studies of Leucocytozoon, Plasmodium, and Haemoproteus from avian blood". *The Journal of Parasitology.* 90 (4): 797-802.

Acknowledgements

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