Surveys

Survey of Haemosporidian Parasites in Resident and Migrant Game Birds of Illinois

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Abstract

Haemosporidian parasites are globally distributed in avian species, and are capable of leading to decreased reproductive success, weakness, and mortality. Bird conservation groups and organizations concerned with the health and immunological status of avian populations are interested in haemosporidian parasites that affect reproduction and population growth. Haemosporidian infection data are not yet always available for some avian species in specific regions. These data provide the starting points for researchers to evaluate geographical and temporal changes in the patterns of infection and prevalence across populations. We examined haemoparasite infections in four game bird species commonly hunted in Illinois. We calculated prevalence, mean intensity, median intensity, and mean abundance of haemosporidians, and evaluated the relation of these infection measures associated with age and sex of the avian hosts. Game species sampled (N = 237) included migrants such as mourning doves Zenaida macroura, wood ducks Aix sponsa, and Canada geese Branta canadensis, as well as resident birds such as wild turkeys Meleagris gallopavo. We identified only Haemoproteus, Plasmodium, and Leucocytozoon species. Haemoproteus was the most prevalent haemosporidian (46/237), followed by Plasmodium (11/237). Furthermore, Haemoproteus was the most persistent haemosporidian, as it was the only parasite genera that we found in all four avian species. We found coinfections in 55% of turkeys, but found no significant correlations between the genera of haemosporidinan coinfections and a host species. Moreover, no significant differences in the proportion of infected individuals (prevalence) and haemosporidian quantities (levels of intensity and abundance) were related to biotic factors such as age and sex of the host. However, parasite aggregation (distribution of parasites among hosts) was affected by age, as adult turkeys and juvenile doves showed the highest aggregation index (Poulin’s index of discrepancy) for Haemoproteus spp. This study reveals patterns of infection and parasite aggregations that vary widely among different game bird species and provides baseline data on avian haemosporidians that, to the best of our knowledge, is not currently available in the state of Illinois for these avian species. Finally, wildlife biologists can use these patterns for management of landscape or host species to support conservation efforts.

Keywords: haematozoan; haemoparasites; Haemoproteus; Leucocytozoon; Plasmodium

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Haemosporidian Parasites in Game Birds of Illinois

K.L. Annetti et al.

Introduction

Haemosporidian parasites affect birds worldwide, wherever suitable vectors are found (Sol et al. 2000). Common genera include \textit{Plasmodium}, \textit{Haemoproteus}, and \textit{Leucocytozoon} (Mullen and Durden 2002; Valkiunas 2004). Haemosporidian infections cause disease and mortality, either indirectly through changes in the behavior of the avian hosts (Asghar et al. 2011; Dunn et al. 2013) or directly due to pathogenicity of the parasite strain and susceptibilities of the avian host (Valkiunas 2004). Factors such as age, immune status, and degree of exposure may contribute to variations in host susceptibility and mortality (Mullen and Durden 2002; Van Wettere 2015).

Clinical signs associated with haemosporidian infections include anorexia, hemolytic anemia, lethargy, and depression, among others (Mullen and Durden 2002; Van Wettere 2015). Postmortem examination may reveal hepatomegaly and splenomegaly (Thrall et al. 2012) caused by the development of different stages of the parasite in the host. Although it is not always possible to observe clinical signs in wild bird species, recent studies have demonstrated that haemosporidian infections in birds are more detrimental than previously thought (Knowles et al. 2010), even at subclinical levels (Dunn et al. 2013). Researchers have used molecular and microscopy tools to evaluate changes in immunological parameters and host fitness associated with blood parasites, and to assess haemosporidians’ impact on population dynamics and life history traits (Asghar et al. 2011; Dunn et al. 2013).

Haemosporidian infections in wild bird populations present a challenge for conservation and avian health. Infections fluctuate through time and space in the host (DeGroote and Rodewald 2010); researchers have found changes in life history traits and population dynamics associated with haemosporidian infections during breeding seasons and at wintering grounds (Asghar et al. 2011; Dunn et al. 2013). Field studies conducted during the breeding season revealed that intensity of haemosporidian infections was associated with reduced energetic condition and late migration timing in some migrants, especially younger birds (DeGroote and Rodewald 2010). Chronic infections contribute to reduced fitness and reproductive success (e.g., hatching and fledging) in breeding birds (Knowles et al. 2010).

Epidemiological surveys and disease surveillance of haemosporidians in wild bird populations are important for the development of effective population management and conservation programs. Such survey efforts serve as early indicators of disease outbreaks and emerging or reemerging diseases that could impact the health of endangered and economically important species. State agencies devote substantial resources to manage the abundance and health of game bird populations for recreational hunting; however, baseline information on haemosporidian infections is lacking in many states. The study of haemosporidians requires knowledge of endemic levels of infection in local host populations to facilitate temporal studies of pathogen diversity, improve detection of new parasites in target species, and evaluate changes in intensity of infections in relation to changes in vector community.

The objectives of this study were to 1) conduct a survey of the haemosporidian species infecting mourning doves \textit{Zenaida macroura} in Champaign County, Illinois; 2) describe parasite infection in mourning doves using prevalence, mean intensity, median intensity, and mean abundance; and 3) explore differences between age and sex associated with measures of infection established in objective 2; these two characteristics have been suggested as factors that contribute to susceptibility of infection in multiple bird species. We hypothesized that these measures of infection would differ between haemosporidian types, and that these differences are associated with the avian host age and sex. Here, we present summary infection data for mourning doves and discuss the benefits of incorporating mean abundance into standard baseline data for haemosporidian studies. In addition, we present summary infection data for three additional game species: wood ducks \textit{Aix sponsa}, Canada goose \textit{Branta canadensis}, and wild turkeys \textit{Meleagris gallopavo}, for which we received incidental samples.

Methods

Field collection

We selected Champaign County, Illinois, as the sampling site for haemoparasite evaluation in mourning doves. Champaign County is characterized by a mixed landscape consisting of primarily cultivated corn and soybean row crops, restored prairies, fragmented forest patches, and residential areas. In addition, over the study period (April to September 2010 and 2011), Illinois hunters and colleagues submitted avian blood samples from commonly hunted game birds to our laboratory. The haemoparasite results for all samples are presented here. We evaluated samples from 19 sites in 15 counties during the study period (Figure 1).

We recorded blood samples and demographic data from mourning doves, wood ducks, Canada goose, and wild turkeys. We evaluated haemosporidian infections in 237 birds (Table 1). We determined age by whether the bird was a hatch year or adult after hatch year. We determined sex of birds where possible based on plumage or physical sexing. We collected blood samples from mourning doves, Canada goose, and wood ducks live trapped by the Illinois Department of Natural Resources as part of a U.S. Fish and Wildlife Service leg band program (Federal Bird Banding and Marking Permit 06507). Prior to their release, we collected a blood sample from the brachial vein following review and approval of the University of Illinois Institutional Animal Care and Use Committee (IACUC 2011) Protocol # 11157. We collected peripheral blood samples from hunter-harvested turkeys (\( n = 20 \)) and wood ducks (\( n = 15 \)) within 4 h postmortem. We analyzed all samples at the Wildlife Veterinary Epidemiology Laboratory at the Illinois Natural History Survey.
Laboratory analysis

For each bird, we made two blood smears, air dried them, fixed and stained them with Diff Quick®. A single observer using a light microscope examined slides. The observer screened each slide for blood parasites at ×400 and ×1,000 until they counted 10,000 red blood cells per slide (Barnard and Bair 1986; Clark et al. 2009). We then stratified data by age, sex, and haemosporidian type.
Table 1. Measures of haemoparasite infection (prevalence, mean intensity, median intensity, and mean abundance) stratified by bird species sampled: mourning doves Zenaida macroura, wood ducks Aix sponsa, Canada geese Branta canadensis, and wild turkeys Meleagris gallopavo. We collected blood samples from 15 counties across the state of Illinois between April and September, 2010 and 2011.a

<table>
<thead>
<tr>
<th>Bird</th>
<th>Parasite species</th>
<th>Infected birds</th>
<th>% Prevalence (lower CI–upper CI)</th>
<th>Mean intensity (lower CI–upper CI)</th>
<th>Median intensity (lower CI–upper CI)</th>
<th>Mean abundance (lower CI–upper CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mourning dove (N = 77)</td>
<td>Haemoproteus sacharovi</td>
<td>30</td>
<td>40.3 (0.292–0.521)</td>
<td>28.19 (9.69–96.5)</td>
<td>4.0 (2.0–7.0)</td>
<td>11.4 (3.94–45.9)</td>
</tr>
<tr>
<td></td>
<td>Haemoproteus columbae</td>
<td>1</td>
<td>60.0 (0.361–0.809)</td>
<td>25.2 (6.08–79.8)</td>
<td>4.0 (1.0–17.0)</td>
<td>15.1 (3.4–53.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>31c</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild turkey (N = 20)</td>
<td>Haemoproteus meleagridis</td>
<td>12</td>
<td>60.0 (0.032–0.379)</td>
<td>25.2 (1.9–4.67)</td>
<td>—ab</td>
<td>4.0 (0.05–1.4)</td>
</tr>
<tr>
<td></td>
<td>Leukocytozoon smithi</td>
<td>3</td>
<td>55.0 (0.315–0.769)</td>
<td>37.5 (18.4–91.8)</td>
<td>25.0 (7.0–39.0)</td>
<td>20.6 (9.45–66.2)</td>
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<tr>
<td></td>
<td></td>
<td>14c</td>
<td></td>
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<tr>
<td>Wood duck (N = 123)</td>
<td>Haemoproteus nettionis</td>
<td>2</td>
<td>—a</td>
<td>—a</td>
<td>—a</td>
<td>—a</td>
</tr>
<tr>
<td>Canada goose (N = 17)</td>
<td>Haemoproteus nettionis</td>
<td>1</td>
<td>—a</td>
<td>—a</td>
<td>—a</td>
<td>—a</td>
</tr>
</tbody>
</table>

a % Prevalence = (no. of infected birds/total no. of birds examined (infected and noninfected) × 100); mean intensity = total no. of parasites within infected host/total no. of infected birds with that parasite; mean abundance = total no. of individual parasites in infected host/total no. of hosts examined (infected and noninfected); confidence intervals for prevalence: Clopper–Pearson; confidence intervals for median intensity: exact confidence limits; confidence intervals for mean intensity and mean abundance: bias-corrected and accelerated bootstrap (BCa Bootstrap).

b CI = 95% confidence intervals.
c Total number of infected birds. Some birds exhibited coinfections.
d Sample too small for estimating this confidence level.
e Two or fewer infected hosts in the sample, CI cannot be calculated.

We applied Spearman’s rank correlation to analyze the interactions between parasites coinfecting the same host, as well as to evaluate the relationship between host characteristics (sex and age) and prevalence, intensity, and abundance (P-value is based on 5,000 Monte Carlo replications; Rózsa et al. 2000). We calculated aggregation index (Polin’s index of discrepancy) by subgroups (age and sex; Table 2). We did not compare infection measures across host species since not all host samples were collected from the same place and time, and the different species studied have different habitat preference and ecology. We considered P < 0.05 significant.

Results

Patterns of haemosporidian prevalence and parasitemia

Haemoproteus, Plasmodium, and Leukocytozoon were the only haemosporidians that we observed in the 237 birds sampled. Representative specimens from infected birds were cataloged at the Harold W. Manter Laboratory of Parasitology (University of Nebraska-Lincoln, Lincoln, Nebraska; accession no. P-2017-021, catalog no. HWML-139028 Haemoproteus nettionis, HWML-139029 Haemoproteus columbae, HWML-139030 Haemoproteus sacharovi, HWML-139031 Haemoproteus nettionis, HWML-139032 Plasmodium hermani and Leukocytozoon smithi, HWML-139033 Haemoproteus meleagridis and Leukocytozoon smithi, HWML-139034 Haemoproteus meleagridis, Plasmodium hermani, and Leukocytozoon smithi). Haemoproteus was the most persistent parasite, as we found it in all four avian host species (Table 1). Mourning doves...
and waterfowls were only infected with Haemoproteus, thus we observed no coinfections in these hosts. Overall, we found coinfections in 55% (11/20) of wild turkeys, with coinfections of H. meleagris–P. hermani (8/20), H. meleagris–L. smithi (1/20), and P. hermani–L. smithi (1/20). Only one turkey (1/20) showed mixed infection with three species of haemosporidians (H. meleagris–P. hermani–L. smithi). From the three haemosporidians found in turkeys, Leucocytozoon was the least prevalent (15%) followed by Plasmodium and Haemoproteus (55 and 60%, respectively). The numbers of turkeys infected with Haemoproteus (n = 12) and Plasmodium (n = 11) were significantly higher when compared to turkeys infected with Leucocytozoon (n = 3; P = 0.0011 and P = 0.0043, respectively). We found no significant correlations between haemosporidian genera coinfecting a host population.

Differences in mean intensity, mean abundance, and haemosporidian infection associated with sex and age

Due to low numbers of infected Canada geese (1/17) and wood ducks (2/123), we did not include waterfowl hosts in these analyses. Wild turkeys and mourning doves were the only hosts that we could analyze for differences in infection measures associated with age. We analyzed differences associated with sex only for mourning doves, because we sampled no female wild turkeys. We analyzed interactions between parasite species coinfecting the same host population only for wild turkeys, since mourning doves were infected only by Haemoproteus parasites. Infection prevalence of Haemoproteus, Plasmodium, and Leucocytozoon in wild turkeys were unassociated with age. Mean intensity and mean abundance were also unassociated with age. We found no significant differences in Haemoproteus prevalence, mean intensity, or mean abundance associated with age and sex in mourning doves. Even though we found no significant differences associated with age and sex of the hosts, the aggregation index categorized by subgroups showed that Haemoproteus parasites were significantly more aggregated in adult-after-hatch-year wild turkeys and hatch-year mourning doves (Table 2).

Discussion

Our results highlight that different infection measures may show different patterns among species, and that these differences carry over to different avian species. Canada geese and wood ducks (N = 140) showed little to no infections with only three (2.14%) waterfowl infected, none of which were infected by Plasmodium or Leucocytozoon parasites. Greiner (1975) reported rates of Plasmodium and Leucocytozoon infection (50% in wood ducks). However, our findings and those of DeJong and Muzzal (2000) show drastically lower infection rates (2.14 and 6.93%, respectively) among waterfowl species. Mourning doves and wild turkeys exhibited greater prevalence, intensity, and abundance than waterfowl host species. Wild turkeys, however, were the only hosts infected with all three parasites (Plasmodium, Haemoproteus, and Leucocytozoon), and the only hosts that showed prevalence above 50% for Haemoproteus and Plasmodium, as well as higher rates of mixed infection (55%) and coinfection with all three observed parasite species (5%). Given that translocation of wild turkeys into new habitats is still occurring, our findings suggest that wild turkeys should be tested for blood protozoans prior to any capture and release efforts to avoid spreading haemoparasites (Forrester 1990).

Differences in prevalence, intensity, and abundance could be influenced by a variety of factors not measured in our study. Ecological, geographical, and avian population characteristics might be responsible for the pattern of infection we observed. For instance, large body size and living in groups or flocks are some of the ecological characteristics that have been suggested as factors that may increase probability of infection.
(Valkiunas 2004) and that could contribute to the patterns of infection found in the turkeys we sampled. Likewise, factors such as numbers of broods per year and time to fledge might have played a crucial role in the dynamics of infection for mourning doves (Valkiunas 2004). Other characteristics, such as nest placement and type of nest, could impact exposure to infection, but in different ways for different host species. For example, previous findings suggest that bird species that typically nest at the mid-understory level show higher prevalence of *Haemoproteus* (González et al. 2014). Prevalence of infection for birds at those nest heights may be related to potential *Haemoproteus* vectors, as some bloodsucking insects such as biting midges and black flies usually preferred mid-understory canopy instead of ground level (Černý et al. 2011). Mourning doves and wood ducks may share a similar range for nest placement; however, type of nest (open and exposed to vectors vs. closed and protected from vectors) could have been one of the contributing factors to infection and prevalence discrepancies between these two bird species.

Multiple factors need to be taken into account in order to better understand probability of infection and overall prevalence of infection for different avian species. Even when living in similar conditions or sharing similar behaviors (e.g., migratory behavior), differences in prevalence and parasitemia may exist. In the case of waterfowl species, a combination of factors such as climate change and food availability could affect migratory behavior and migration range (Rodewald 2015). When bird migration behaviors change, such as those of resident Canada geese that no longer migrate north to breed in Canada, interactions with specific parasites and vectors might decrease, impacting and potentially explaining our results (lower prevalence of infection: 2.14% waterfowls vs. 40.26% mourning doves). We recognize that when dealing with migrant species, such as mourning doves, we likely had a mixed sample of migrants and residents in our sampled birds. This mixed sample of hosts from potentially two different geographical locations would have resulted in exposure to potentially different vectors involved in the transmission of haemosporidian parasites. Therefore, we are unable to conclude that the infection patterns in mourning doves were a reflection of local infection pathways.

Contradictory results in haemosporidian prevalence associated with sex and age have been reported in other bird studies. Some researchers indicated greater prevalence of infection in adults, others in young birds, and a third group found no differences between age groups (Greiner 1975; Godfrey et al. 1990; Valkiunas 2004). Similarly, there are reported differences in the association of parasite prevalence with sex (Fedynich and Rhodes 1995; McCurdy et al. 1998; Asghar et al. 2011). We found no significant differences in prevalence, intensity, or abundance associated with age and sex in any host. However, significant differences in aggregation index (Table 2), as identified in our study, showed that parasite aggregation patterns are different for different factors (age vs. sex) and in different host species (mourning doves vs. wild turkeys). Our results suggest that biotic factors such as age play an important role in parasite loads, but the effect is not the same for all avian host and haemosporidian species.

Our small, male-only sample for turkeys (N = 20), prevented us from evaluating inferences related to sex, and the hunter harvest samples and timing of sampling (e.g., hunting seasons) may have biased our samples to be all male. Increasing the frequency of sampling within an area, increasing the number of avian samples collected, evaluating the vector composition by sampling site, and stratifying the sampling areas by forest, agricultural or grassland sites, would help to improve our understanding of the epidemiology and distribution of haemosporidian infections in Illinois game birds. Hunter harvest samples continue to be a powerful source of samples for haemoparasite studies in avian game species. Future work should focus on specific areas or evenly sampled target counties, as well as in identifying and analyzing other parasites (ecto- and/or endoparasites) or viruses that could affect prevalence of haemosporidians and overall transmission dynamics (Forrester 1990; Mbah et al. 2014; Medeiros et al. 2014).

Changes in the abundance of one parasite can influence coinfection and competition among other parasites, potentially altering susceptibility to disease within the host (Telfer et al. 2010; Johnson and Hoverman 2012), and disease dynamics among hosts, and by proxy affecting the exposure to ecological pressures outside the host (Bouwman and Hawley 2010; Hawley and Altizer 2011, Hawley et al. 2011). Mean abundance is a measure reported in this study that highlights the dispersion of a parasite among the entire population, noninfected individuals included. It is possible to observe high intensity among infected individuals but a low abundance in the overall population. In our data, *Haemoproteus* exhibit these different aspects of abundance vs. intensity in turkeys and doves. Wild turkeys showed relatively high abundance and intensity of infections while doves showed higher intensity but lower abundance (Table 1). A population with relatively low abundance of infection may not be as much of a concern compared to a population with a few intensely infected individuals. A few intensely infected individuals may serve as an effective parasite reservoir and a source of infection for vectors, and in turn for naïve birds (Woolhouse et al. 1997; VanderWaal and Ezenwa 2016). Within a population, host infection with high intensity and abundance could possibly alter host fitness, immune status, and reproductive patterns, ultimately affecting host behavior (Hawley and Altizer 2011). Parasites in wild turkey populations in the state of Illinois should be further analyzed in order to better understand the impact of vector abundance, parasite interactions and infection risk (Telfer et al. 2010), transmission among hosts (Mbah et al. 2014; Medeiros et al. 2014), and changes in behavior that may alter disease dynamics (Bouwman and Hawley 2010; Hawley et al. 2011).

Overall, our results are in agreement with previous reports. Cross-immunity does not appear to develop in wild birds infected with haemosporidians; we found coinfection (in 55% of all birds sampled) with multiple
parasites from *Haemoproteus*, *Plasmodium*, and *Leucocytozoon* species. Our results also indicate that haemosporidians have host fidelity, with specificity associated by bird order (e.g., the Anatidae family was the only family of birds with *H. nettionis*). Furthermore, more than one haemosporidian species belonging to one genus can infect the same host species (Valkiunas 2004). The distribution of haemosporidians among game bird species in Illinois varied widely among host species. The variables influencing the epidemiology of haemosporidian infection in Illinois game birds and the impact of haemosporidian infections on avian populations continue to be understudied. We can improve our understanding of the epidemiology and distribution of avian hemoparasitic infection by evaluating changes in vector abundance and parasite infection, the biotic factors associated with vertebrate and invertebrate hosts, and the abiotic factors (environmental climate variables and landscape characteristics) that contribute to haemosporidian infection patterns in host species.

**Archived Material**

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Table A1. Sex, age, and number of haemosporidian parasites (*Haemoproteus*, *Plasmodium*, and *Leucocytozoon*) identified in blood samples collected across the state of Illinois between April and September, 2010 and 2011. We sampled four bird species: mourning doves *Zenaida macroura*, wood ducks *Aix sponsa*, Canada geese *Branta canadensis*, and wild turkeys *Meleagris gallopavo*.

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