HOST RANGE OF *MYCOPLASMA GALLISEPTICUM* IN EASTERN NORTH AMERICA

A Thesis

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by

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ABSTRACT

Mycoplasma gallisepticum, an avian pathogen most common in poultry, was first detected in wild songbirds in 1994, primarily infecting house finches (*Carpodacus mexicanus*) in which it can cause severe eye lesions. Recent studies have revealed that *M. gallisepticum* can infect a greater diversity of avian hosts. Our study attempts to determine the host range of *M. gallisepticum* in a bird community in Tompkins County, New York (USA). This research was conducted between January 2007 and June 2010 as part of a larger *M. gallisepticum* investigation. We tested to what extent bird taxonomic affiliation, and seasonal presence influenced the likelihood of being infected. Birds were trapped opportunistically at bird feeders and inspected visually for conjunctivitis. Conjunctival swabs were tested for the presence of *M. gallisepticum* DNA using polymerase chain reaction (PCR); blood samples were tested for the presence of *M. gallisepticum*-specific antibodies using rapid plate agglutination (RPA). The 1,989 individuals sampled comprised 53 species from 19 avian families. We documented evidence of *M. gallisepticum* infection in 27 species from 15 avian families. Overall, 37/1989 (1.9%) of the individuals showed visible signs of conjunctivitis, with 77/1989 (3.9%) testing positive for *M. gallisepticum* via PCR, and 72/1989 (3.6%) testing positive for *M. gallisepticum* antibodies via RPA. Overall, 58/1056 (5.5%) fringillids tested positive via PCR, with 40/331 (12.1%) positive results from house finches specifically, and 18/933 (1.9%) from non-fringillids generally. We found positive PCR and RPA results in 11 species of migratory birds, and no evidence of *M. gallisepticum* infection in 26 of the species sampled (n=57). Successful isolates of the bacteria were made from seven field samples. When combining the results from this study with previous research, there is evidence of *M. gallisepticum* infection in 42 bird species.

BIOGRAPHICAL SKETCH

Jonathan DeCoste became interested in birds when he enrolled in an ornithology class as a senior at Skidmore College studying environmental biology. Little did he know that this course would set him on a career path in the field of avian biology. After completing his bachelor's degree, Jonathan embarked on the requisite journey of low-paying, highly adventurous field jobs studying birds around the world. It wasn't long before he was offered a job as the field technician on the House Finch Project at the Cornell Laboratory of Ornithology. Jonathan's thesis comes from data collected while doing field work for this project.

This thesis is dedicated to Dr. Corey Freeman-Gallant, whose passion and love of birds opened my eyes to the wonders of the avian world; and whose trust and friendship allowed me to explore that world and make it my career.

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Introduction

Mycoplasmal conjunctivitis was first observed in 1994 in the Washington D.C. area (Fischer et al., 1997; Dhondt et al., 1998). The disease, caused by a novel strain of *Mycoplasma gallisepticum*, primarily infects house finches (*Carpodacus mexicanus*) in which it can cause severe eye lesions (Ley et al., 1996). This small passerine, native to the western United States, is also present across the eastern part of the continent following an introduction by the pet industry in 1940. The epidemic spread quickly throughout the eastern population of house finches resulting in significant population declines (Dhondt et al., 1998; Hochachka and Dhondt, 2000). Although a well-documented bacterial pathogen in poultry, this marked one of the first instances that *M. gallisepticum* was isolated in wild songbirds (Ley et al., 1997).

One of the major complexities in the dynamics of *M. gallisepticum* in house finches is the role that other host species might play in local prevalence and transmission. Since the initial outbreak in 1994, research has shown that species other than house finches may suffer from the same conjunctival disease, or at least have detectable levels of *M. gallisepticum*. One of the first documented outbreaks of conjunctivitis in species other than house finches occurred during the winter of 1998-1999 in Quebec, Canada. Researchers documented numerous cases of conjunctivitis caused by *M. gallisepticum* in evening grosbeaks (*Coccothraustes vespertinus*) and pine grosbeaks (*Pinicola enucleator*) (Mikaelin et al., 2001). Since that time, field investigations of wild birds in North America have detected *M. gallisepticum* in seven other bird species, while reports from the House Finch Disease Survey and other studies documented over 30 species showing signs of conjunctivitis (Table 1; Hartup

Table 1: Summary of *M. gallisepticum* infection in wild birds across all studies.

Species with reported conjunctivitis, positive PCR and serology results

Family	Species	Serology	*M. gallisepticum* infection[x,y]	Conjunctivitis Reported
Cardinalidae	Northern Cardinal	**3/28[a], 5/49[b], 33/157[c]**	**1/28[a,x]**, 0/6[c,y]	**Yes[d]**
Columdidae	Mourning Dove	0/3[a], **3/54[b]**, 0/4[c]	**3/54[b]**	**Yes[d]**
Emberizidae	American Tree Sparrow	**2/46[a]**, 0/15[d]	**1/46[a,x]**	**Yes[d]**
Emberizidae	Song Sparrow	**7/121[a]**, 0/3[b], **1/58[c]**, 0/1[d]	**1/121[a]***	**Yes[d]**
Emberizidae	White-throated Sparrow	**1/21[a]**, 0/27[b], **11/91[c]**, 0/10[d]	**1/21[a,x]**, 0/3[c,y]	**Yes[d]**
Fringillidae	American Goldfinch	**8/537[a], 6/41[b], 3/97[c], 1/9[d]**	**15/537[a,x]**	**Yes[a,d]**
Fringillidae	House Finch	**11/337[a], 30/112[c], 4/23[d]**	**40/331[a,x]**, **6/84[c,y]**	**Yes[a,b,c,d,f]**
Fringillidae	Pine Siskin	**3/154[a]**, 0/1[c]	**2/154[a,x]**	**Yes[d]**
Fringillidae	Purple Finch	0/28[a], 0/21[c], **3/24[d]**	**1/28[a,x]**, **1/5[d]**	**Yes[a]**
Icteridae	Red-winged Blackbird	0/74[a], **1/1[b]**, 0/2[c]	**3/74[a,x]**	**Yes[d]**
Paridae	Eastern Tufted Titmouse	**5/36[a], 4/17[b], 32/44[c], 4/8[d]**	0/36[a,x], **12/28[c,y]**	**Yes[d]**
Picidae	Downy Woodpecker	**1/36[a]**	**1/36[a,x]**	**Yes[d]**

Species without reported conjunctivitis and positive PCR and serology results

Family	Species	Serology	*M. gallisepticum* infection[x,y]	Conjunctivitis Reported
Emberizidae	White-crowned Sparrow	**1/23[a]**	**1/23[a,x]**	–
Passeridae	House Sparrow	**6/111[a]**, **6/33[b]**, 0/24[c]	**1/111[a,x]**	–
Turdidae	Wood Thrush	**4/5[a]**	**1/5[a,x]**	–

Species with reported conjunctivitis and positive serology results

Family	Species	Serology	*M. gallisepticum* infection[x,y]	Conjunctivitis Reported
Cardinalidae	Rose-breasted Grosbeak	**1/9[a]**,	0/9[a,x]	**Yes[d]**
Corvidae	Blue Jay	0/9[a], **1/3[b]**, 0/5[c]	–	**Yes[d]**
Emberizidae	Chipping sparrow	0/6[a], **4/20[b], 4/88[c]**	0/1[c,y]	**Yes[d]**
Icteridae	Brown-headed Cowbird	**1/11[a], 1/7[b], 14/19[c], 2/6[d]**	0/11[a,x], 0/3[c,y]	**Yes[d]**
Icteridae	Common grackle	0/6[a], **8/15[c]**	0/3[c,y]	**Yes[d]**
Paridae	Black-capped Chickadee	**12/160[a]**	0/160[a,x]	**Yes[a,d]**

Sturnidae	European Starling	**1/31**[c]	0/160[a,x]	**Yes**[d]
Trogolodytidae	Carolina Wren	0/6[b], **3/2**[c]	0/160[a,x]	**Yes**[d]
Turdidae	American Robin	**3/19**[a], 0/2[b], **10/16**[c]	0/160[a,x]	**Yes**[d]

Species with reported conjunctivitis and positive PCR results only

Family	Species	Serology	*M. gallisepticum* infection[x,y]	Conjunctivitis Reported
Bombycillidae	Cedar Waxwing	0/10[a]	**1/10**[a,x]	**Yes**[d]
Fringillidae	Evening Grosbeak	_	**YES**[e,y]	**Yes**[b,d]
Fringillidae	Pine Grosbeak	_	**YES**[e,y]	**Yes**[b]

Species with positive PCR results only

Family	Species	Serology	*M. gallisepticum* infection[x,y]	Conjunctivitis Reported
Emberizidae	Dark-eyed Junco	0/15[a], 0/37[c], 0/5[d]	**1/15**[a,x]	**Yes**[d]
Parulidae	Common Yellowthroat	0/13[a]	**1/13**[a,x]	_
Sittidae	Red-breasted Nuthatch	0/2[a]	**1/2**[a,x]	_
Tyrannidae	Eastern Phoebe	0/3[a], 0/9[c]	**1/3**[a,x]	_
Tyrannidae	Traill's Flycatcher	0/6[a]	**1/6**[a,x]	_
Vireonidae	Red-eyed Vireo	0/3[a]	**1/3**[a,x]	_

Species with positive serology results only

Family	Species	Serology	*M. gallisepticum* infection[x,y]	Conjunctivitis Reported
Emberizidae	Field Sparrow	**3/79**[c]	_	_
Emberizidae	Savannah sparrow	0/2[a], **2/25**[c]	_	_
Icteridae	Northern Oriole	**1/9**[a]	0/9[a,x]	_
Mimidae	Gray Catbird	0/45[a], **2/2**[b]	_	_
Mimidae	Northern mocking bird	**3/11**[b], **2/17**[c]	_	_
Mimidae	Brown thrasher	0/1[a], **4/9**[b], 0/7[c]	_	_
Paridae	Carolina Chickadee	**2/17**[b], **2/14**[c]	0/1[c,y]	_
Parulidae	Pine Warbler	**1/1**[c]	_	_
Parulidae	Yellow-rumped warbler	0/27[b], **9/21**[c]	0/1[c,y]	_

Table 1(Continued)

Table 1 (Continued)

Species with reported conjunctivitis only

Family	Species	Serology	*M. gallisepticum* infection[x,y]	Conjunctivitis Reported
Cardinalidae	Lazuli Bunting	–	–	Yes[d]
Columdidae	Rock Dove	–	–	Yes[d]
Fringillidae	Common redpoll	0/6[a]	0/6[a]	Yes[d]
Laniidae	Northern Shrike	–	–	Yes[d]
Sittidae	White-breasted nuthatch	0/19[a]	0/19[a]	Yes[d]
Trochilidae	Ruby-throated hummingbird	–	–	Yes[d]
Turdidae	Eastern bluebird	0/2[a], 0/7[c]	–	Yes[d]

Species with no reported conjunctivitis or detected *M. gallisepticum*

Family	Species	Serology	*M. gallisepticum* infection[x,y]	Conjunctivitis Reported
Cardinalidae	Dickcissel	0/1[c]	–	–
Cardinalidae	Indigo Bunting	0/5[a]	–	–
Columdidae	Eurasian Collared Dove	0/1[b]	–	–
Emberizidae	Eastern towhee	0/7[b], 0/7[c]	–	–
Emberizidae	Fox Sparrow	0/3[c]	–	–
Emberizidae	Lincoln's sparrow	0/1[a]	–	–
Emberizidae	Swamp sparrow	0/1[a], 0/1[b]	–	–
Fringillidae	Field Sparrow	0/2[a]	–	–
Fringillidae	Fox Sparrow	0/5[a]	–	–
Hirundinidae	Tree swallow	0/1[a]	–	–
Parulidae	American redstart	0/1[a]	0/1[a]	–
Parulidae	Black and white warbler	0/1[a]	0/1[a]	–
Parulidae	Yellow-breasted Chat	0/2[b]	–	–
Picidae	Hairy woodpecker	0/4[a], 0/1[c]	–	–
Picidae	Red-bellied woodpecker	0/6[c]	–	–
Picidae	Yellow-bellied sapsucker	0/1[a]	–	–

Regulidae	Golden-crowned kinglet	0/5[b]		–	–
Regulidae	Ruby-crowned kinglet	0/1[a], 0/9[b], 0/4[c]		–	–
Sittidae	Brown-headed nuthatch	0/3[c]		–	–
Trogolodytidae	House Wren	0/1[b]		–	–
Trogolodytidae	Winter wren	0/1[a]		–	–
Tyrannidae	Acadia flycatcher	0/2[a]	0/2[a]		–
Tyrannidae	Eastern wood peewee	0/1[a]		–	–
Tyrannidae	Least flycatcher	0/2[a]		–	–
Tyrannidae	Willow flycatcher	0/1[a]		–	–
Vireonidae	Yellow-throated vireo	0/1[a]		–	–

[a:] **This Study**. Sampled for the bacteria in both eyes of all species trapped via PCR; sampled for antibodies via RPA.

[b:] **Farmer et al., 2005**. Sampled for the bacteria the choanal cleft of all species trapped via PCR; sampled for antibodies via Serum Plate Agglutination (SPA).

[c:] **Luttrell et al., 1996, 2001**. Focused sampling on fringillids, birds with visible eye lesions, and birds associated with poultry facilities via PCR in both eyes. Used positive SPA results for sampling asymptomatic individuals. Also used histopathology as a detection method.

[d:] **Hartup et al., 2000, 2001**. Focused sampling on fringillids and birds with visible eye lesions via PCR in infected eyes. Sampled the right eye of asymptomatic individuals. Sampled for antibodies using SPA.

[e:] **Mikaelian et al., 2001.** Sampled necropsied individuals with visible signs of conjunctivitis. Used PCR and histopathology.

[f:] **Ley et al., 1996, 1997.** Cultured field samples to obtain isolates.

[x]: Results by PCR

[y]: PCR results obtained from necropsy

Table 1 (Continued)

et al., 2000, Hartup et al., 2001; Luttrell et al., 2001; Farmer et al., 2005). Given these findings, there is a need for additional investigation into whether *M. gallisepticum* disease dynamics in other species are closely linked to those in house finches. Specifically, we need to know if conjunctivitis in house finches is part of an interconnected multi-host system.

While attempts have been made to determine the host reservoir for *M. gallisepticum*, initial studies reported the bacteria primarily in house finches and other fringillid species (Hartup et al., 2000; Hartup et al., 2001; Luttrell et al., 2001; Farmer et al., 2005). Often, these studies failed to sample all the individuals trapped, opting to focus primarily on house finches (Luttrell et al., 1996; Hartup et al., 2001), birds with visible eye lesions (Hartup et al., 2000; Luttrell et al., 2001), and birds with strongly seropositive blood samples (Luttrell et al., 2001). The only study to sample every bird of all species caught sampled for the bacteria only in the choanal cleft, a location where the bacteria is routinely detected in poultry but where it is only occasionally detected in infected house finches (Farmer et al., 2005; Sydenstricker et al. 2006; unpublished data). Failing to sample for *M. gallisepticum* in the conjunctiva, where it is most commonly found in house finches, may cause cases of infection to go undetected. Given the large geographic range that this epidemic now covers, it is likely that there are more species acting as a reservoir of *M. gallisepticum* than have been documented (Dhondt et al., 1998; Hochachka and Dhondt, 2000). Research on West Nile virus has shown that, while many species may be infected with the virus, only a relative few may be competent hosts and able to spread the epidemic (Kilpatrick et al., 2006). Within such epidemics, there can be extreme heterogeneity

in transmission that arises from differences in host infectiousness (Woolhouse et al., 2001; Kilpatrick et al., 2006). The habitat use and spatial distributions of host species, along with variation in susceptibility, immunological competence, and species diversity are all important factors in pathogen transmission (Dobson, 2004).

The objective of this study was to determine the extent to which wild birds were infected with *M. gallisepticum* and the possibility of there being a large previously undetected host reservoir for the bacteria. We will be addressing the following four questions in this paper. 1). How widespread is *M. gallisepticum* in the community of birds that associate with house finches and bird feeders? 2). Is there any clear taxonomic pattern to *M. gallisepticum* presence in wild birds? 3). How does seasonal variation in the prevalence of *M. gallisepticum* in host species compare to that seen in house finches? 4). Are there any important life-history traits of infected host species that are associated with the presence of *M. gallisepticum*? To answer these questions, we trapped and sampled wild birds for *M. gallisepticum* over 2.5 years throughout Tompkins County, NY. By combining our results with those previously published, we provide a more detailed list of free-living hosts of *M. gallisepticum.*

Materials and Methods

The study was conducted between January 2007 and June 2010 in Tompkins County, New York (42°46' N, 76° 45' W). Wild birds were trapped using mist nets and cage traps under New York State Fish and Wildlife License 39 (Albany, NY) and permit 22669 from the United States Geological Survey, Department of the Interior (Laurel, MD). All sampling procedures were approved by Cornell University's Institutional Animal Care and Use Committee (permit 2006-094). Several locations were used for year-round sampling throughout Tompkins County. At each site, bird-feeding stations were maintained continuously, using black oil sunflower seeds. All birds trapped opportunistically at the feeding stations were sampled for *M. gallisepticum*.

All birds in the study were marked with a unique aluminum leg band (USGS) at the time of initial capture. The weight (g), tarsus length (mm), and wing chord length (mm) were recorded for each individual. The body condition (0-5 scale) and furcular fat deposits (0-5 scale) were also scored. We looked for physiological differences between PCR positive and PCR negative individuals using a standard t-test. The eyes and conjunctiva were scored for disease on a 0 to 3 scale, where a bird with no visible signs of conjunctivitis has a score of 0 and a bird with extreme conjunctival eye lesions has a score of 3 (Sydenstricker et al., 2006). After the examination, all individuals were swabbed in each eye using separate Puritan polyester tipped aluminum swabs, and both swabs were used to inoculate Frey's bacterial growth media (Ley et al., 1996) whether disease symptoms were present or not. Additionally, a blood sample was taken from the brachial vein using a 27 gauge ½" hypodermic needle and a heparinized capillary tube for serology screening. Blood

samples were immediately put on ice following sampling. Within 24 hours of sampling, serum was extracted from blood samples in the lab using a 12,000 rpm micro-hematocrit centrifuge and tested for *M. gallisepticum* antibodies via rapid plate agglutination (RPA) and a commercially available *M. gallisepticum* antigen from Charles River Laboratories, Inc. Presence or absence of *M. gallisepticum* antibodies was recorded as such.

From 2007-2009, individual eye swabs were tested for *M. gallisepticum* via polymerase chain reaction (PCR). At that time, no attempts at isolates were made. From 2009-2010, field samples taken using Frey's media were incubated for one week to assure bacterial growth. One 25 microliter aliquot of the medium was divided into three samples to be tested for the presence of *M. gallisepticum* DNA using end-point PCR methods (Geary et al., 1994). All samples were initially screened with 16s rRNA gene primers (Lauerman, 1998). Starting in 2009, any samples that were *M. gallisepticum* positive after the initial screening were tested again using the MGC2 house finch strain specific *M. gallisepticum* primer. Prior to 2009, attempts were made at culturing all positive 16s samples for *M. gallisepticum* isolation. After 2010, only samples testing positive with the MGC2 primers were used for culture and isolation. The study switched from Frey's media to Copan Universal Transport Medium in 2010 because of difficulty obtaining *M. gallisepticum* isolates from PCR positive field samples.

Results

3.1 Host range

Among the 1,989 individuals trapped between 2007 and 2010 we found evidence for infection with *M. gallisepticum* in 27 of 53 species sampled belonging to 19 avian families: we observed conjunctivitis in four species; *M. gallisepticum* DNA was detected in conjunctival swabs of 20 species using PCR; and *M. gallisepticum*-specific antibodies were identified in sera of 18 species. Only in the two species with the largest sample sizes (house finch, n=537, American goldfinch n=331) all three criteria for *M. gallisepticum* infection were found (Table 2). Among symptomatic birds, 31/37 (83.8%) were house finches and 4/37 (10.8%) were American goldfinches. One purple finch and one black-capped chickadee also showed signs of conjunctivitis. Overall, 77/1989 (3.9%; 19 species) of samples tested positive for *M. gallisepticum* DNA using the 16s PCR primers (Table 2). Ten species that tested positive by PCR using the 16s primers never showed any clinical symptoms, although it must be noted that we were only able to detect *M. gallisepticum* in one individual of many of these species (Table 2). 47/72 (65.3%) of RPA positive results occurred in eleven species that also tested positive for *M. gallisepticum* DNA with the 16s primers. 12/77 (15.6%) of 16s positive *M. gallisepticum* results occurred in nine species that never tested positive for *M. gallisepticum* antibodies. 24/77 (31.2%) of positive *M. gallisepticum* antibody results occurred in seven species that never tested positive for *M. gallisepticum* DNA. We were unable to detect any evidence of *M. gallisepticum* infection in 26 of the species we sampled, although it should be noted that eleven of these species are represented by only one individual (Table 1).

Table 2: Positive PCR and RPA results of feeder and non-feeder birds in Tomkins County, NY, 2007-2010

Family	Species	Number Sampled	PCR Positive	RPA Positive	Conjunctivitis
Feeder Birds					
Fringillidae	American Goldfinch (*Carduelis tristis*)	537	15 (2.8%)	8 (1.5%)	4 (0.07%)
Fringillidae	House Finch (*Carpodacus mexicanus*)	331	40 (11.9%)	11 (3.3%)	31 (9.2%)
Fringillidae	Pine Siskin (*Carduelis pinus*)	154	2 (1.8%)	3 (2.7%)	0
Fringillidae	Purple Finch (*Carpodacus purpureus*)	28	1 (3.6%)	0	1 (3.6%)
Cardinalidae	Northern Cardinal (*Cardinalis cardinalis*)	28	1 (3.6%)	3 (10.7%)	0
Cardinalidae	Rose-breasted Grosbeak (*Pheucticus ludovicianus*)	9	0	1 (11.1%)	0
Emberizidae	American Tree Sparrow (*Spizella arborea*)	46	1 (2.2%)	2 (4.4%)	0
Emberizidae	Dark-eyed Junco (*Junco hyemalis*)	15	1 (6.7%)	0	0
Emberizidae	Song Sparrow (*Melospiza melodia*)	121	1 (0.8%)	7 (5.8%)	0
Emberizidae	White-crowned Sparrow (*Zonotrichia leucophrys*)	23	1 (4.4%)	1 (4.4%)	0
Emberizidae	White-throated Sparrow (*Zonotrichia albicollis*)	21	1 (4.8%)	1 (4.8%)	0
Icteridae	Brown-headed Cowbird (*Molothrus ater*)	11	0	1 (9.1%)	0
Icteridae	Red-winged Blackbird (*Agelaius phoeniceus*)	74	3 (4.1%)	0	0
Paridae	Black-capped Chickadee (*Poecile atricapilla*)	160	0	11 (6.9%)	1 (0.01%)
Paridae	Eastern Tufted Titmouse (*Baeolophus bicolor*)	36	0	5 (13.9%)	0
Passeridae	House Sparrow (*Passer domesticus*)	111	1 (0.9%)	6 (5.4%)	0
Picidae	Downy Woodpecker (*Picoides pubescens*)	36	1 (2.8%)	1(2.8%)	0
Sittidae	Red-breasted Nuthatch (*Sitta canadensis*)	2	1 (50.0%)	0	0
Non-Feeder Birds					
Bombycillidae	Cedar Waxwing (*Bombycilla garrulus*)	10	1 (10%)	0	0
Icteridae	Northern Oriole (*Icterus galbula*)	9	0	1 (11.1%)	0
Mimidae	Gray Catbird (*Dumetella carolinensis*)	45	0	3 (6.7%)	0
Parulidae	Common Yellowthroat (*Geothlypis trichas*)	13	1 (7.7%)	0	0
Turdidae	American Robin (*Turdus migratorius*)	19	0	3 (15.8%)	0
Turdidae	Wood Thrush (*Hylocichla mustelina*)	5	1 (20.0%)	4 (80.0%)	0
Tyrannidae	Eastern Phoebe (*Sayornis phoebe*)	3	1 (33.3%)	0	0
Tyrannidae	Traill's Flycatcher (*Empidonax traillii*)	6	1 (16.7%)	0	0
Vireonidae	Red-eyed Vireo (*Vireo olivaceus*)	3	1 (33.3%)	0	0

3.2 *M. gallisepticum* and feeder use

Detection of *M. gallisepticum* via PCR in species with different feeding strategies varied only slightly in our study. We found that birds most commonly associated with bird feeders tested positive 70/1708 (4.1%) times, while birds not typically associated with bird feeders tested positive for the bacteria 6/113 (5.3%) times (Table 2). Using a general linear model, we found no statistical significance in the coefficients of *M. gallisepticum* infected feeder vs. non-feeder birds (-3.1990, SE=0.1211; -2.7175, SE=0.3902).

3.3 Taxonomic patterns to *M. gallisepticum* infection

We detected *M. gallisepticum* in a large diversity of species, although visible signs and detection of the bacteria were still most prevalent in fringillid birds. 36/37 (97.3%) cases of observed conjunctivitis were recorded in resident species of the Fringillidae. Generally, 58/1056 (5.5%) fringillid birds were PCR positive at the time of sampling. Within those results, 40/331 (12.1%) house finches tested PCR positive. We also documented 22/1056 (2.1%) positive RPA results in fringillid species. Our study captured an eruption of pine siskins, and while none of the 154 individuals we sampled showed any signs of conjunctivitis, we detected *M. gallisepticum* via PCR using 16s primers in two individuals, and *M. gallisepticum* antibodies in three other individuals via RPA. Conversely, 18/933 (1.9%) non-fringillid birds were PCR positive at the time of sampling, with 39/933 (4.2%) non-fringillid birds testing RPA positive. We also documented *M. gallisepticum* infection via PCR in one northern cardinal. In the Paridae family, only one black-capped chickadee was observed to

have mild eye lesions. However, both PCR and RPA results for this individual were negative. Overall, we were unable to detect *M. gallisepticum* via PCR in any birds in this family, although we did have many RPA positive results for both eastern tufted-titmice and black-capped chickadees [(5/36 (13.9%) and 11/160 (18.3%) respectively)].

Of all 1,989 individuals sampled, we recaptured 187 individuals. Of those individuals, 13 were house finches and 28 were goldfinches. Although none of the goldfinches were PCR or RPA positive at either capture event, several house finches differed in disease status between captures. Three individuals were PCR and RPA negative at the initial capture, and either PCR positive, RPA positive, or both at the second capture event. One individual went from being PCR positive at the initial sampling to PCR negative in the eight months between capture events, and one individual remained PCR positive at both sampling events two weeks apart. The other eight birds had no detectable *M. gallisepticum* at either sampling events. We also recaptured seven individual black-capped chickadees on at least one other occasion. Of these recaptures, three individuals had positive serologic results at least once. One individual was positive on the initial capture date and negative when recaptured six and seven months later. One individual was captured four times over a month and a half, testing positive two weeks apart on the last two sampling occasions. The final individual was captured four times over a nearly two month period, testing positive on the second sampling occasion one week after the first, and negative 19 days later.

3.4 Seasonality of *M. gallisepticum* infections

Detected disease prevalence varied systematically across seasons in both house finches and more generally in species in which *M. gallisepticum* infection was identified using PCR. These raw *M. gallisepticum* prevalence data were grouped by month across all years and then analyzed separately to test for possible seasonal variation in *M. gallisepticum* prevalence among house finches, resident species, summer migrants, and winter migrants (Figure 1). Among house finches, there is a clear fall peak in raw prevalence in September and October with 10/77 (13.0%) and 6/50 (12.0%) birds testing positive via PCR for *M. gallisepticum* respectively. This is followed by a second larger peak in late winter, with 20/63 (31.8%) house finches testing positive for the bacteria in February (Figure 1). Among resident species, there is a low continual prevalence of *M. gallisepticum* throughout the year (2.1% average), with August being the only month where *M. gallisepticum* was not detected (n=88). Similar to the house finch double peak in the fall and then again in late winter, October and February are the only months were *M. gallisepticum* is detected in winter migrants, 1/5 (20.0%) and 3/109 (2.8%) respectively. We also saw *M. gallisepticum* in summer migrants sampled in May, June, and July; 1/51 (2.0%), 2/39 (5.1%), and 2/35 (5.7%) respectively.

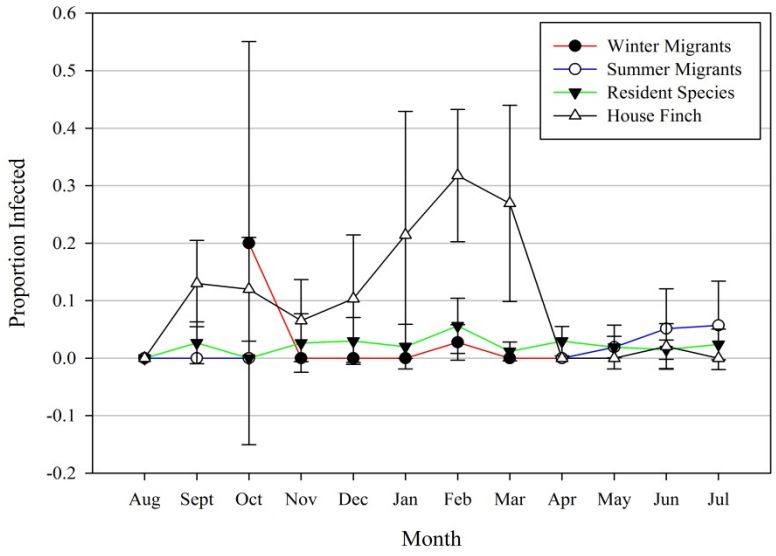

Figure 1. Monthly prevalence (mean +/- 1SE) of *M. gallisepticum* infection across bird species with differing migration strategies. Results obtained by PCR.

3.5 *M. gallisepticum* and life history strategies

Among the 11 non-resident bird species that tested positive for *M. gallisepticum* there exist three migration strategies. Eight are neotropical migrants that overwinter in Central and South America and breed in North America; two are arctic migrants that arrive in Tompkins County during the winter months and migrate north during the spring and summer. White-crowned sparrows, finally, pass through Tompkins County during the fall and spring migration

3.6 *M. gallisepticum* and phenotypic variation

The standard phenotypic measurements taken did not document any significant differences in weight, fat nor body condition between PCR positive and PCR negative

house finches (t-test; t_{40}=2.02, P=0.095; t_{41}=2.02, P=0.26; t_{39}=2.02, P=0.12 respectively).

3.7 Culture of *M. gallisepticum* field samples

Between 2009 and 2011, we attempted to make isolates from 51 PCR positive *M. gallisepticum* field samples. 15 *M. gallisepticum* positive samples were cultured in Frey's media, tested for *M. gallisepticum* via PCR using the 16s primers and then frozen at -80°C. These 15 samples (13 house finches, one American goldfinch, and one downy woodpecker) yielded no *M. gallisepticum* isolates. The remaining 36 samples were tested using Copan Universal Transport Medium (one common redpoll, one purple finch, 34 house finches). These samples were taken from all birds trapped on days when we caught symptomatic birds, whether they had eye lesions or not. We successfully cultured seven field isolates of *M. gallisepticum*, all from symptomatic house finches.

Discussion

4.1 Host range

We sampled 53 avian species during the course of this study, 23 of which had not been sampled in other studies. Although this covers a larger number of species than previously sampled, it is far from comprehensive as the number of bird species regularly occurring in New York State is well over 400 (Levine, 1998). We have combined the results for all 75 species sampled by us and others in Table 1. In 42 of 75 species (56.0 %) evidence for infection by *M. gallisepticum* was detected. This suggests that there may be a much larger host reservoir for *M. gallisepticum* than previously documented (Hartup et al., 2000), and even than found in this study. While visible signs of the disease were most apparent in house finches, we identified a large number of asymptomatic bird species that could play a role in transmission of *M. gallisepticum*. When combining all studies of conjunctivitis and of *M. gallisepticum*, it becomes clear that conjunctivitis had been previously reported in many of the species that we found to be PCR and/or RPA positive in our study but asymptomatic at the time of sampling (Table 1; Hartup et al., 2000; Hartup et al., 2001; Mikaelian et al., 2001). This supports the idea that conjunctivitis in these species could result from *M. gallisepticum* infection, rather than from conjunctivitis-causing infections such as avian pox.

4.2 *M. gallisepticum* is detected both in feeder and in non-feeder species

Among the 42 species across all studies in which evidence of *M. gallisepticum* infection was found, 26 of the species were birds that come regularly to bird feeders

(Table 1). In our study alone, 18 of the species in which evidence of *M .gallisepticum* was found are birds that come regularly to bird feeders (Table 2). Given that *M. gallisepticum* remains infectious on bird feeders up to 24 hours, these results should not be surprising (Dhondt et al., 2007). Infected birds could be shedding the bacteria on the surface of bird feeders and infecting any subsequent individuals feeding at that location. That feeders play a role in transmission is supported by the case of a blue jay (*Cyanocitta cristata*) contracting *M. gallisepticum* after being housed in a cage that previously housed disease house finches (Ley et al., 1997). Infected feeder birds included a downy woodpecker and northern cardinals (*Cardinalis cardinalis*), a species that had previously only tested positive via serology, although there was observed conjunctivitis and histologic lesions (Table 1; Farmer et al., 2005; Luttrell et al., 2001). The cardinal cases are interesting as States et al. (2009) demonstrated that conjunctivitis prevalence in house finches increased when northern cardinal numbers were higher. Other common feeder bird species that routinely showed evidence for *M. gallisepticum* infection are parids. Combining our results with those from other studies black-capped chickadees (antibodies in 12/160), Carolina chickadees (antibodies in 4/31), and Eastern tufted titmice (antibodies in 45/105; DNA in 12/64) are frequently infected (Farmer et al., 2005; Hartup et al., 2000; Luttrell et al., 2001). Additionally, 3 of 74 red-winged blackbirds tested positive for *M. gallisepticum* via PCR (Table 2). Of the three, two tested positive in July and one tested positive in April, possibly implicating another reservoir species for the bacteria during the breeding season in the northern hemisphere.

The 16 species from eight avian families that rarely associate with bird feeders in which we found evidence for *M. gallisepticum* infection (Table 1, Table 2) are species of thrushes, flycatchers, warblers, vireos, blackbirds, mimics, waxwings, and wrens (Table 1, Table 2). This shows that *M. gallisepticum* is not limited to feeder birds or birds regularly coming into contact with house finches. Therefore, there may be modes of transmission that are maintaining *M. gallisepticum* in wild birds that cannot be explained by bird feeders contaminated with the bacteria or by direct contact with diseased house finches.

4.3 Seasonality of *M. gallisepticum* infections

Our study found strong seasonal peaks in *M. gallisepticum* infection in house finches as well as a small year round reservoir of the bacteria in other species based on PCR results (Figure 1). These strong peaks of infection that occur in fall and late winter in house finches confirm earlier work that found similar seasonal results, attributing them to larger numbers of susceptible juvenile birds in the fall, and stressful conditions late in winter (Hartup et al., 2001; Altizer et al., 2004; Jennelle et al., 2007). Interestingly, there seems to be a small continually maintained reservoir for the bacteria circulating in resident species as well as in winter and summer migrants. This low-level maintenance may be the source for the reoccurring outbreaks of the disease in house finches, especially given the low detection rate of the bacteria during the breeding season.

4.4 Life history strategies of bird species where *M. gallisepticum* was detected

The life history strategies of several of the PCR and RPA positive bird species may have important implications in the transmission of this disease. 11 species of migratory birds are represented in these positive data. A possible consequence of these migratory species testing positive for *M. gallisepticum* bacteria is an increased likelihood of the pathogen spreading southward along the migration corridors from North America through Central and South America as well as northward into the arctic.

4.5 Potential pitfalls: potential false negatives and false positive results

Although in 26 species we did not detect evidence for *M. gallisepticum* infection this could be the result of the small sample sizes for many of these species. In all, except the yellow-rumped warbler (n=48), Eastern towhee (n=14), and ruby-crowned kinglet (n=14) 5 or fewer individuals were sampled. Larger samples might show *M. gallisepticum* infections in these species also.

As others previously, we used the 16s primer to detect *M. gallisepticum* DNA. This primer is known to also react to *M. gypsis* and *M. imitans* (Ley et al., 2010), which might have generated some false positives. That is why later in the study we also used the MGC2 primer for PCR analyses. This primer is specific for the house finch *M. gallisepticum* strain (Ley et al., 2010).

Further influencing our ability to detect *M. gallisepticum* in the wild may be differences in the encounter rates of healthy versus infected individuals. Based on previous Capture-Mark-Recapture studies, encounter rates of individuals infected with

M. gallisepticum can be lower than those of non-infected birds, resulting in an underestimation of infection prevalence (Jennelle et al., 2007). It is unclear, however, if encounter rates also differ between non-infected and asymptomatic infected individuals.

Whereas captive experiments with infected house finches have shown the RPA test to be a reliable means for detecting *M. gallisepticum* antibodies in house finches (Kollias et al., 2004), serologic results in previous field studies, as well as our own, may be reporting higher positive *M. gallisepticum* antibody test results. Feberwee et al. (2005) demonstrated that there is both a higher chance of false positive results and the possibility of the antigen reacting with *M. gallisepticum*, *M. synoviae,* and *M. imitans* when using undiluted serum samples. Unfortunately, due to the realities of field sampling, it is impossible to obtain enough blood to run the RPA test with diluted samples. In nine species, the presence of antibodies was the only evidence for infection by *M. gallisepticum* (Table 2).

Conclusions

The scope of these data, even when considering the issues with the primers and serology, give important insight into the natural history of *M. gallisepticum*. Because our earliest samples were tested using a less specific but more sensitive primer we may have inadvertently stumbled upon an important clue to the emergence of this infectious disease. Since *M. gallisepticum* is a well-studied bacterial pathogen in poultry, the long-standing belief was that the bacteria made the jump from poultry to wild birds. There are many strains of *Mycoplasma gallisepticum*, including the house finch strain, widely circulating in numerous bird species that have a diverse variety of migration and feeding strategies (Geary et al., 1994; Ley et al., 1997). These other bird species with evidence of *M. gallisepticum* infection have the potential for being both the source of the initial outbreak of the bacteria in house finches, as well as being a constant source for re-exposure and infection from non-fringillid species (Lovette, pers. com.). It may even be the case that the strain of *M. gallisepticum* that is so virulent to house finches was commonly circulating in North American bird species long before the outbreak of the disease in the mid-1990s. Widespread detection of *M. gallisepticum* in other species could indicate that the bacteria has been widely and benignly present in these bird populations before any evidence of disease was discovered, and whether through an increase in virulence or a chance exposure to the historically western house finch, the epidemic erupted. In the future, the culture and sequencing of these non-house finch specific strains of *M. gallisepticum* needs to be a

priority in order to determine where these bacteria fall in the relationship to poultry and house finch strains.

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