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## MYCOPLASMAL CONJUNCTIVITIS IN SONGBIRDS FROM NEW YORK

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**ABSTRACT:** A field study was conducted to determine the prevalence of conjunctivitis and *Mycoplasma gallisepticum* (MG) infections in house finches (*Carpodacus mexicanus*) and other songbirds common to bird feeders in Tompkins County (New York, USA). Eight hundred two individuals of 23 species and nine families of birds were captured and given physical examinations during the 14 mo study beginning in February 1998. Clinical conjunctivitis (eyelid or conjunctival swelling, erythema, and discharge) was observed in 10% (19/196) of house finches examined, and only in the winter months from November to March. Unilateral conjunctivitis was observed in 79% (15/19) of affected house finches; one case developed bilateral disease between 8 and 18 days following initial examination. Conjunctivitis was observed in a similar proportion of males and females sampled, and body condition scores and wing chord lengths were not significantly different between diseased and non-diseased house finches. *Mycoplasma gallisepticum* was isolated from 76% (13/17) of finches with conjunctivitis and 2% (3/168) of clinically normal house finches sampled during the study. DNA fingerprints of 11 MG isolates using random amplification of polymorphic DNA (RAPD) techniques showed no apparent differences in banding patterns over the course of the study, suggesting persistence of a single MG strain in the study population. The prevalence of conjunctivitis and MG infections declined in house finches between February/March 1998 and February/March 1999 (23% to 6%, and 20% to 5%, respectively), but only the former was significant ( $P < 0.05$ ). Conjunctivitis was also observed in four American goldfinches (*Carduelis tristis*) and one purple finch (*Carpodacus purpureus*). *Mycoplasma gallisepticum* infection was confirmed in the purple finch, the first documented case of MG-associated conjunctivitis in this species. The purple finch isolate was similar to house finch isolates from the study site by RAPD analysis. Positive plate agglutination (PA) tests were recorded in one other goldfinch and two purple finches, suggesting exposure of these individuals to MG. Positive PA tests were also obtained from two brown-headed cowbirds (*Molothrus ater*) and four tufted titmice (*Parus bicolor*), but MG infection could not be confirmed in these cases due to lack of samples. Based on these findings, the prevalence of MG infections in hosts other than house finches appear to be low in the population sampled. There is growing evidence, however, that songbird species other than house finches are susceptible to MG infection and disease.

**Key words:** *Carpodacus mexicanus*, *Carpodacus purpureus*, conjunctivitis, host range, house finch, *Mycoplasma gallisepticum*, mycoplasmosis, purple finch.

### INTRODUCTION

*Mycoplasma gallisepticum* (MG) infections are associated with conjunctivitis in house finches (*Carpodacus mexicanus*) and American goldfinches (*Carduelis tristis*) in eastern North America (Fischer et al., 1997; Ley et al., 1996, 1997). Despite the persistence of the epidemic in house finches for more than 5 yr, no field investigations have been undertaken to determine the prevalence of conjunctivitis and MG infections in this species. The single published field study on conjunctivitis in

house finches was designed to determine the etiology of the disease by selectively sampling diseased house finches from affected populations (Luttrell et al., 1996). Dhondt et al. (1998) used a citizen-survey to successfully describe the spread of the epidemic and the proportion of bird feeding stations with diseased house finches, but could not provide population-based estimates of disease prevalence. We believe that a suite of studies are required to fully understand the epidemiology of this emergent disease, from broad-scale surveys to more traditional field investigations

which will link survey data with localized patterns of disease.

In conjunction with longitudinal studies of conjunctivitis and MG infections in house finches, there is the need to assess the frequency of disease in other potential host species. Only a small number of MG isolations have been made from goldfinches to date (Ley et al., 1997). An MG isolate was obtained from a blue jay (*Cyanocitta cristata*) early in the course of this epidemic, but the bird was believed to have acquired a nosocomial infection at a rehabilitation facility (Ley et al., 1996). Porter (1994) described a similar clinical syndrome in purple finches (*Carpodacus purpureus*) from Virginia in 1994, but did not culture MG from them. A recent study suggested MG exposure in several other songbird species, most notably the tufted titmouse (*Parus bicolor*), but lack of MG isolations from any case raised concern over the validity of the serological test used in these species to indicate exposure to MG (P. Luttrell, pers. comm.). Survey results on purple finches, house sparrows (*Passer domesticus*), dark-eyed juncos (*Junco hyemalis*) and black-capped chickadees (*Parus atricapillus*) revealed a low occurrence of conjunctivitis in these species compared to house finches through October 1996 (Hartup et al., 1998). These studies suggest MG infections and conjunctivitis are rare in songbirds other than house finches, and are unlikely to represent a limiting factor for these species' populations. Continued disease surveillance, however, may be useful in detecting changes in the host-parasite relationship that may result in significant population impacts, especially parasite adaptation to hosts with declining populations or known susceptibility to MG.

The objectives of this study were to document the occurrence of mycoplasmal conjunctivitis in house finches common to residential feeding stations, and to conduct opportunistic sampling of other selected songbird species for conjunctivitis and evidence of MG exposure or infection.

## MATERIALS AND METHODS

The study was conducted between February 1998 and March 1999 in Tompkins County (New York, USA; 42°44' N, 76°50' W). Birds were captured with traps or mist nets under permits from the New York State Department of Environmental Conservation (Albany, New York, USA) and the United States Fish and Wildlife Service, Department of Interior (Washington D.C., USA). Traps and nets were distributed among bird feeders maintained year-round at five residential sites. Sampling efforts were concentrated on species of the Family Fringillidae (house finches, purple finches and American goldfinches), while members of other families were sampled on a more opportunistic basis. The length of the study was chosen to increase the probability of gaining access to several irruptive finch species, such as common redpolls (*Carduelis flammea*) and evening grosbeaks (*Coccothraustes vespertinus*), that may have otherwise been left unsampled over a single winter.

All birds were identified by applying a numbered aluminum leg band (Bird Banding Laboratory, Laurel, Maryland, USA). Additionally, house finches were marked with individual combinations of plastic, colored leg bands between November 1998 and March 1999 (Avinet, Inc., Dryden, New York, USA). All birds were given a physical examination that included close inspection of the eyes and adnexa for signs of conjunctivitis (eyelid or conjunctival swelling, erythema, exudation or epiphora). Wing chord length (mm) and a body condition score (1 to 5 scale where 1 has no visible furcular fat deposits and little pectoral muscle mass and 5 has extensive furcular fat deposits with rounded pectoral muscle mass) were also determined. Birds that were sampled had the right eye (if normal) or an affected eye swabbed for mycoplasma identification by culture or PCR, and/or a blood sample taken from the right jugular vein for mycoplasma serology prior to release.

Both active and passive searches for follow-up of color-banded house finches were conducted beginning in November 1998. Active searches were done weekly for 4 to 6 hours by the primary author at each of the banding sites. Passive searching consisted of opportunistic observations of banded house finches throughout each week both at banding locations and elsewhere throughout Tompkins County by interested amateur and professional ornithologists. In all cases, observations consisted of a color band combination and sex description for positive identification of individuals, as well as a clinical evaluation of each eye and adnexa for signs consistent with conjunctivitis.

Conjunctival swabs taken in the field were immediately immersed in mycoplasma broth and incubated later that same day according to the protocol described by Hartup and Kollias (1999). Mycoplasma colonies on agar media were identified by direct immunofluorescence (Talkington and Kleven, 1983). Aliquots of broth cultures from all birds were tested for the presence of MG-specific DNA by polymerase chain reaction (PCR) methods (Lauerman, 1998). Twelve *Mycoplasma gallisepticum* isolates made during the study were later compared using a random amplification of polymorphic DNA (RAPD) technique (Ley et al., 1997) using two primer systems (Geary et al., 1994; Fan et al., 1995). Each RAPD assay included DNA extracts from a historical house finch MG isolate and one or more MG vaccine strains used in commercial poultry (F, 6/85, ts-11) for comparison. Blood samples were placed immediately into lithium heparin tubes and placed on ice (Microtainer, Becton-Dickinson, Rutherford, New Jersey, USA). Plasma was tested later the same day for antibodies to MG by the plate agglutination test (PA; Kleven and Yoder, 1989), using a scoring system similar to Luttrell et al. (1998).

Disease frequency data is presented as the proportion of individuals with conjunctivitis, MG infection (positive culture or PCR test) or a positive PA test among individuals sampled during the 14-month study. In addition, the prevalence of conjunctivitis and MG infections was stratified by month for house finches between November 1998 and March 1999. Both capture and re-sighting data were used to estimate the prevalence of conjunctivitis in house finches for this period, while MG infection prevalence could only be determined from captured individuals. The body condition scores and wing chord lengths of house finches with conjunctivitis and MG infections were compared to those of healthy and uninfected house finches, respectively, using logistic regression (Hosmer and Lemeshow, 1989). Sex and the month of capture were included as potential confounding variables in each analysis (BMDP statistical software, version 7.0, Los Angeles, California, USA). Potential associations between sex and conjunctivitis or MG infection status were evaluated with chi-square tests. The prevalence of conjunctivitis and MG infections in house finches for the two-month periods February/March 1998 and February/March 1999 were also compared using Fisher's exact test (EpiInfo v. 6.04, 1997 version, Centers for Disease Control and Prevention, Atlanta, Georgia, USA). Statistical significance was established at  $P < 0.05$ .

## RESULTS

Eight hundred two individuals of 23 species and nine avian families were examined during this study (Table 1). Two hundred and eight recaptures of various species and 233 resightings of color-banded house finches provided a total of 1,243 observations of birds common to bird feeders in Tompkins County for physical evidence of conjunctivitis.

Conjunctivitis was observed in 10% (19/196) of the house finches sampled during the study, either at initial capture or during follow-up. Diseased house finches were only observed between the winter months of November and March at our sites. Monthly conjunctivitis prevalence ranged from 5% (3/66) to 8% (5/61) during the winter of 1998–99 (Fig. 1). A smaller sample from March 1998 revealed 17% (3/18) of house finches affected with conjunctivitis. The prevalence of conjunctivitis in house finches declined between February/March 1998 (23%, 5/22) and February/March 1999 (6%, 5/81,  $P < 0.05$ ).

Eighty four percent (16/19) of diseased house finches exhibited unilateral conjunctivitis. Two individual birds initially observed with unilateral conjunctivitis had follow-up available: one bird's gross appearance remained unchanged for at least 12 days, and the other developed bilateral conjunctivitis between 8 and 18 days after initial examination. Overall, conjunctivitis was observed in a similar proportion of males and females sampled ( $P > 0.05$ ). Mean body condition scores and wing chord lengths were not different between diseased and non-diseased house finches ( $P > 0.05$ ).

*Mycoplasma gallisepticum* was isolated from 16 house finches during the study (3 were clinically normal, 13 exhibited conjunctivitis). In addition, one clinically normal and culture negative house finch was positive for MG by PCR testing, providing a total of 17 infected house finches. One of the four infected disease-free finches was re-sighted approximately 2 mo later and appeared normal. The outcomes of

TABLE 1. Clinical and diagnostic findings from songbird species evaluated for conjunctivitis and *Mycoplasma gallisepticum* infections in Tompkins County (New York, USA).

Family	Species	Conjunctivitis	MG infection <sup>a</sup>	Serology <sup>b</sup>
Cardinalidae	Northern cardinal ( <i>Cardinalis cardinalis</i> )	0/2 <sup>c</sup>	— <sup>d</sup>	—
Cardinalidae	Rose-breasted grosbeak ( <i>Pheucticus ludovicianus</i> )	0/1	—	—
Corvidae	Blue jay ( <i>Cyanocitta cristata</i> )	0/1	—	—
Emberizidae	American tree sparrow ( <i>Spizella arborea</i> )	0/105	0/2	0/15
Emberizidae	Chipping sparrow ( <i>Spizella passerina</i> )	0/12	—	—
Emberizidae	Dark-eyed junco ( <i>Junco hyemalis</i> )	0/26	—	0/5
Emberizidae	Song sparrow ( <i>Melospiza melodia</i> )	0/7	—	0/1
Emberizidae	White-throated sparrow ( <i>Zonotrichia albicollis</i> )	0/27	—	0/10
Fringillidae	American goldfinch ( <i>Carduelis tristis</i> )	4/169 (2%)	0/53	1/9 (11%)
Fringillidae	Common redpoll ( <i>Carduelis flammea</i> )	0/6	—	—
Fringillidae	Evening grosbeak ( <i>Coccothraustes vespertinus</i> )	0/11	—	0/8
Fringillidae	House finches ( <i>Carpodacus mexicanus</i> )	19/196 (10%)	17/194 (9%)	4/23 (17%)
Fringillidae	Pine siskin ( <i>Carduelis pinus</i> )	0/2	—	—
Fringillidae	Purple finch ( <i>Carpodacus purpureus</i> )	1/45 (2%)	1/5 (20%)	3/24 (13%)
Icteridae	Brown-headed cowbird ( <i>Molothrus ater</i> )	0/11	—	2/6 (33%)
Icteridae	Common grackle ( <i>Quiscalus quiscula</i> )	0/3	—	—
Icteridae	Red-winged blackbird ( <i>Agelaius phoeniceus</i> )	0/11	—	—
Paridae	Black-capped chickadee ( <i>Parus atricapillus</i> )	0/137	—	—
Paridae	Tufted titmouse ( <i>Parus bicolor</i> )	0/14	0/2	4/8 (50%)
Picidae	Downy woodpecker ( <i>Picoides pubescens</i> )	0/3	—	—
Sittidae	Red breasted nuthatch ( <i>Sitta canadensis</i> )	0/3	—	—
Sittidae	White-breasted nuthatch ( <i>Sitta carolinensis</i> )	0/6	—	—
Turdinae	American robin ( <i>Turdus migratorius</i> )	0/4	—	—

<sup>a</sup> Positive MG culture or PCR test.<sup>b</sup> Plate agglutination test.<sup>c</sup> Positive/observed or tested.<sup>d</sup> Not tested.

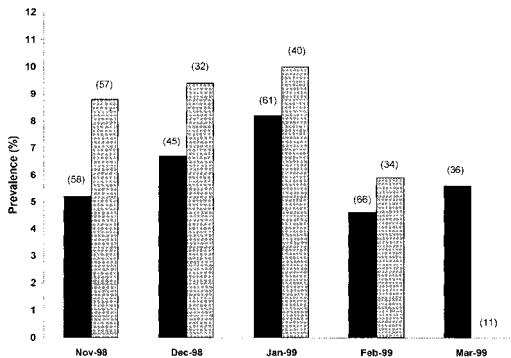


FIGURE 1. Monthly prevalence of conjunctivitis (black bars) and MG infection determined by culture or PCR (gray bars) in Tompkins County house finches during the winter of 1998–99. Numbers in parentheses are the sample size on which the prevalence estimate is based.

the other cases are unknown. The monthly prevalence of MG infections ranged from 0% (0/11) to 10% (4/40) during the winter of 1998–99 (Fig. 1). A small sample from March 1998 revealed 27% (3/11) of house finches infected with MG. Though the prevalence of MG infections in house finches of the study area declined between February/March 1998 (20%, 3/15) and February/March 1999 (4%, 2/45), this difference was not statistically significant ( $P > 0.05$ ).

DNA fingerprints of 11 house finch MG isolates showed no apparent differences in RAPD banding patterns over the study period (Figs. 2, 3). The DNA profiles were also similar to two historic isolates from North Carolina and Kentucky (USA) made early in the epidemic, but were different from three MG vaccine strains. These relationships were later confirmed by using a second primer set for RAPD analysis (Fan et al., 1995; data not shown).

Conjunctivitis was documented in two species other than house finches during the study. One male and two female American goldfinches were observed with unilateral eyelid and conjunctival swelling, epiphora and mild nasal discharge. One female goldfinch exhibited bilateral conjunctivitis. The cases were observed in March 1998 ( $n = 2$ ), May 1998 ( $n = 1$ ), and Jan-

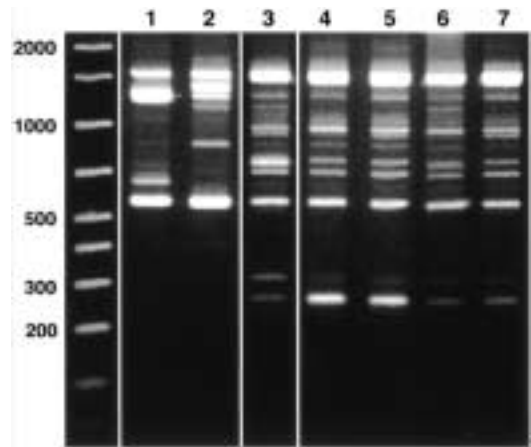


FIGURE 2. RAPD patterns of MG vaccine strains and isolates from songbirds using the Geary et al. (1994) primer 1254. Lane 1 shows vaccine strain ts-11, lane 2 shows vaccine strain 6/85, lane 3 is for 1996 house finch isolate #K4117 from Kentucky, lanes 4–6 are February and March 1998 Tompkins County house finch isolates, and lane 7 is an April 1998 Tompkins County purple finch isolate. DNA base pair size standards are shown on the left of each gel (AmpliSize Molecular Ruler, Bio-Rad Laboratories, Hercules, California, USA). Samples not relevant to the present study have been removed with the aid of photo editing software (Photoshop 5.0, Adobe Systems Inc., San Jose, California, USA).

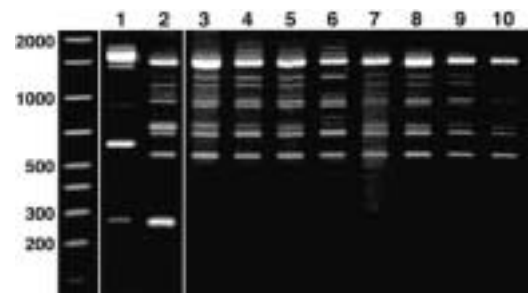


FIGURE 3. RAPD patterns of MG vaccine strains and isolates from songbirds using the Geary et al. (1994) primer 1254. Lane 1 represents vaccine strain F, lane 2 is the 1994 house finch isolate #7994 from North Carolina, and lanes 3–10 are from Tompkins County house finch isolates made between November 1998 and February 1999. DNA base pair size standards are shown on the left of each gel (AmpliSize Molecular Ruler, Bio-Rad Laboratories, Hercules, California, USA). Samples not relevant to the present study have been removed with the aid of photo editing software (Photoshop 5.0, Adobe Systems Inc., San Jose, California, USA).

uary 1999 ( $n = 1$ ). Diagnostic testing of the three goldfinches with unilateral conjunctivitis failed to demonstrate MG infection either by isolation or PCR testing. No diagnostic testing was performed on the goldfinch with bilateral conjunctivitis.

An adult female purple finch was captured in April 1998 with unilateral eyelid swelling and epiphora. A conjunctival swab yielded MG growth after 20 days incubation. A PCR test of the culture media confirmed the presence of MG DNA in the sample. A RAPD fingerprint of the isolate from this bird showed marked similarity to several early 1998 MG isolates from Tompkins County house finches and one historical house finch isolate (Fig. 2). The bird was released after sampling, but no follow-up information is yet available on this case.

Positive PA tests were obtained from 17% (4/23) of blood samples collected from house finches. Four of five house finches with conjunctivitis yielded a positive PA test. The single positive PA test from a goldfinch was observed in a clinically normal individual. Additionally, the female purple finch described above was positive for antibodies to MG by the PA test. No other goldfinches or purple finches surveyed for exposure to MG by this method yielded positive findings. Positive PA tests were observed in two brown-headed cowbirds (*Molothrus ater*) and four tufted titmice. All six of these birds were clinically normal. No culture or PCR data, however, are available from these cases to help determine their infection status. Opportunistic serological testing among small samples of American tree sparrows (*Spizella arborea*), dark-eyed juncos, a song sparrow (*Melospiza melodia*), white-throated sparrows (*Zonotrichia albicollis*) and evening grosbeaks revealed no positive PA tests.

#### DISCUSSION

The results of this study revealed a low prevalence of mycoplasmal conjunctivitis among wintering house finches from a northeastern population. We were limited

by the availability of house finches visiting bird feeders at different times of the year; our ability to detect comparatively rare MG infections and make valid estimates of disease prevalence was compromised by spring dispersal of breeding finches as early as March in both years. Though the disease was not observed during the intervening breeding season at our banding sites, several cases of mycoplasmal conjunctivitis were diagnosed among wild adult and juvenile house finches from Tompkins County presented for rehabilitation during summer and fall 1998. Our estimates of conjunctivitis prevalence in this population are similar to those from a revised citizen-survey using bird count data that covered New York (USA) during the same time period (unpubl. data). There appears to be considerable variation in disease prevalence, however, between local northeastern sites with wintering house finches. At our second field site in Mercer County, New Jersey, monthly conjunctivitis prevalence among house finches ranged from two percent (January, 1999) to 40% (February, 1999) during the winter of 1998–99 (unpublished data). These prevalence patterns suggest a dynamic host-parasite relationship that depends on numerous factors to produce localized epidemics, such as shifts in population density (tendency to form winter flocks), feeding behavior (reliance on bird feeders) and environmental stressors (winter storms and cold stress). Additional field and laboratory investigations are needed to elucidate the critical factors responsible for these variations in disease frequency.

To date, studies of house finches in captivity suggest the disease may have a profound impact on host survival due to severe morbidity and high mortality (Luttrell et al., 1998). Our field study cannot confirm or refute the prevailing impression that conjunctivitis negatively affects host survival. In our study, one finch observed with conjunctivitis was followed for 1 mo with no resolution of clinical disease. Alternatively, one infected individual ap-

peared clinically normal 2 mo after diagnosis. Generally low recapture rates and a limited sample of diseased house finches has restricted our ability to estimate survival probabilities in this open population using standard capture-recapture techniques. Continued marking of house finches with colored bands and re-sightings may provide these estimates in the near future.

Based on physical examinations, free-ranging house finches with mycoplasmal conjunctivitis at bird feeders appear to be in good body condition despite locally severe clinical disease. We lack information, however, on the pathogenesis of the disease in most of the infected finches, and so cannot directly compare our findings with those of Luttrell et al. (1998) which showed steady declines in weight and body condition over several weeks after onset of clinical disease in captive house finches. Additionally, we found a preponderance of cases of unilateral conjunctivitis in house finches at bird feeders, similar to Luttrell et al. (1998). Interestingly, 81% (13/16) of these cases were limited to the left eye. Our findings also suggest there is no sex bias in diseased house finches or a pattern of susceptibility to disease in larger individuals as suggested by Nolan et al. (1998).

The estimates of MG infection prevalence parallel those of conjunctivitis. The greater frequency of MG infections compared to conjunctivitis from November 1998 to February 1999 is likely due to the presence of birds in the early stages of infection or potentially in an infected, but disease-free (e.g., lacking conjunctivitis or upper respiratory disease) carrier state (Luttrell et al., 1998). Prevalent cases of conjunctivitis remained in the population in March 1999, but there appeared to be a decrease in the frequency of MG infections. This decrease may have been due to arrival of migrant, trap-naïve disease-free house finches, dispersal of winter flocks that may have lowered transmission rates among susceptible hosts and hence disease prevalence, or may be a function of small sample size that lowered the probability of

detecting an infected house finch. Though the prevalence of both conjunctivitis and MG infections appeared to decrease from February/March 1998 to February/March 1999, only the former was statistically significant. Continued longitudinal sampling is necessary to determine whether the disease has reached an endemic equilibrium or is cycling within the population.

The DNA fingerprints of isolates made during this study suggest persistence of a single strain of MG in Tompkins County house finches. This strain retains considerable similarity to historical house finch isolates from two distant sites, and yet remains different from MG vaccine strains, supporting the findings of Ley et al. (1997). We did not document any marked alterations in disease prevalence suggesting the emergence of a new strain or change in the parasite's virulence during this study. At present, the RAPD technique appears to be the most useful molecular epidemiological tool for monitoring this disease in songbirds.

We also observed clinical conjunctivitis in American goldfinches and purple finches during this study. The disease prevalence, however, was at levels considerably less than that observed in house finches from the same area. Infection with the house finch strain of MG was confirmed in a single purple finch, and exposure to MG suggested in two additional purple finches and one goldfinch by positive PA tests. We believe these cases represent spillover of MG infections from house finches. As predicted elsewhere, MG may be transmitted between house finches and other host species by direct contact or via contamination of bird feeders (Hartup et al., 1998).

Positive serological tests were observed in brown-headed cowbirds and tufted titmice during the study. Our study lacks culture and PCR data for these cases because of the study's design; we cannot conclusively confirm or refute these findings. Yet, these findings may have resulted from non-specific serological reactions and be in error. The validity of the PA findings in



these species are uncertain because of the test's potential for false positives and its lack of validation in these two non-domestic avian species (Kleven and Yoder, 1989). In addition, P. Luttrell (pers. comm.) noted similar findings in several songbird species, most notably tufted titmice, but was unable to demonstrate MG infections with culture, though positive PCR tests were associated with several individuals. Exposure of these species to MG could also occur at bird feeding stations. In addition, Hartup and Kollias (1999) have hypothesized that exposure of cowbirds to MG may occur in parasitized nests of house finches where infected adult and nestling house finches may transmit pathogenic mycoplasmas to developing cowbird chicks. Additional investigation on the potential expansion of host range in these species is warranted.

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