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Effects of *Mycoplasma gallisepticum* on Reproductive Success in House Finches

Paul M. Nolan,[A] Sharon R. Roberts, and Geoffrey E. Hill

Department of Biological Sciences, 331 Funchess Hall,
Auburn University, Auburn, AL 36849

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SUMMARY. Long known as a pathogen of poultry, *Mycoplasma gallisepticum* (MG) was first detected in house finches in 1994. The disease rapidly spread throughout the eastern United States and Canada and was associated with debilitating disease and high mortality in house finches. However, in the late 1990s, the proportion of infected finches dying as a result of infection with MG decreased, and asymptomatic infection was more common among wild birds than in the past. We documented MG infections in breeding house finches and concluded that adults of both sexes transmit the infection to dependent young, probably after hatch. MG infections of breeding adults occurred late in the breeding season and were found in birds completing significantly more nests than birds that never tested positive for MG, implying that higher rates of reproduction carry a cost in the form of increased risk of infection. We found evidence of an MG-induced delay in dispersal of nestlings from their natal area and demonstrated a significant impact of infection on nestling growth.

RESUMEN. Efectos del *Mycoplasma gallisepticum* en el comportamiento reproductivo de los pinzones comunes.

El *Mycoplasma gallisepticum* (MG), reconocido desde hace tiempo como un microorganismo patógeno en la industria avícola, fue detectado por primera vez en pinzones comunes en el año 1994. La enfermedad se diseminó rápidamente en el occidente de Estados Unidos y Canadá, presentando las aves infectadas un cuadro de debilidad y altos niveles de mortalidad. Sin embargo, en los últimos años de la década de 1990, el porcentaje de mortalidad en los pinzones infectados con MG disminuyó y las infecciones asintomáticas fueron cada vez más comunes en estas aves. Estudiamos las infecciones por MG en la reproducción de pinzones comunes y concluimos que las aves adultas de ambos sexos son capaces de transmitir la enfermedad a los pinzones jóvenes anidados, probablemente después del nacimiento. La infección por MG en pinzones adultos ocurre tarde en la temporada de apareamiento y es más común en aves que completan nidadas en porcentajes significativamente mayores, en comparación con aves no infectadas. Esto implica que el éxito reproductivo conlleva un riesgo mayor de infección. Encontramos evidencias de que la infección causa un retardo en el tiempo requerido por los pichones para abandonar los nidos y se demostró un impacto negativo en el crecimiento de los mismos.

Key words: *Mycoplasma gallisepticum*, house finch, vertical transmission, pseudovertical transmission, conjunctivitis, disease

Abbreviations: MG = *Mycoplasma gallisepticum*; PCR = polymerase chain reaction; SPA = serum plate agglutination assay

Diseases can have large impacts on the development and reproductive success of wild birds and may even affect the dynamics of avian populations (14). The extent of a disease outbreak's impact depends, in part, on the method of its transmission and on the severity of its effects on infected individuals. Evidence of a changing relationship between the house finch (*Carpodacus mexicanus*) and its recently established pathogen, *Mycoplasma gallisepticum* (MG), raised the possibility that infections were occurring among breeding finches. We tested for the prevalence and effects of such infections and investigated the possibility of

[A]Corresponding author. Current address: School of Life Sciences, P.O. Box 874501, Arizona State University, Tempe, AZ 85287.

vertical transmission of the parasite (i.e., vertical transmission of MG to hatchlings via the egg).

Mycoplasma gallisepticum is known primarily as a pathogen of domestic poultry (13), with few records of its occurrence in songbirds. Beginning in 1994, MG was responsible for an outbreak of conjunctivitis in wild house finches in the mid-Atlantic United States (5). Since then, MG has spread throughout the eastern range of the house finch, reaching epidemic levels in east-central Alabama in the summers of 1996–98 (3,5,21). From 1994–98, tens of millions of house finches are believed to have died in this ongoing outbreak of mycoplasmal conjunctivitis (17). After the initial outbreak in 1994/1995, infection rates at our study site in Alabama peaked in the summer of 1996 and have steadily declined since then.

Luttrell et al. (16) described the progression of MG through a captive flock of house finches in 1995/1996 and found that a high percentage of the birds rapidly developed debilitating illnesses that often led to death. In a similar study in 1998/1999, asymptomatic birds showing detectable levels of antibodies to MG infected a captive, seronegative flock (22). Nearly all the house finches in that flock became infected with MG and showed clinical signs of mycoplasmal conjunctivitis, but none died from the disease. Furthermore, a significant subset of birds in this latter study remained chronically infected but asymptomatic for the duration of the study. Similarly, Roberts et al. (21) detected a number of infected but asymptomatic house finches in a wild population between March 1998 and February 1999, the period immediately preceding the 1999 breeding season. These observations suggest that asymptomatic infected adults breed in the wild.

In September 1996–98, immediately after completion of the breeding seasons in those years, prevalence of mycoplasmal conjunctivitis on our study site reached peaks of 60%, 23%, and 21%, respectively (17,21). By the start of the breeding season (mid-February through mid-August) of 1999, birds in our population seemed able to survive MG infection, increasing the possibility of infected birds breeding and transmitting the infection to their offspring. Transmission of MG from adults to their offspring occurs in poultry by direct, vertical transmission (20,23) and also in a manner described by Bencina et al. (1) as pseudovertical transmission, or infection of dependent young after they hatch. Hartup and Kollias (6) surveyed house finch eggs and nestlings in 1998 and found only a small incidence of mycoplasmal infection in choanal and conjunctival swab samples pooled within broods. However, their pooling of samples prevented analysis of MG's effects on individuals, and their lack of access to data describing nestling development and adult infection status and subsequent reproductive success prompted us to investigate the possibility of vertical transmission of MG in our population.

We tested for the possibility of vertical transmission of MG, which has been reported for poultry. We also assessed both the prevalence of mycoplasmal infection in a breeding house finch population and its effects on the reproductive success and return rates of breeding birds. Finally, we assessed the prevalence of MG infection among nestlings and tested for effects of infection on nestling condition.

MATERIALS AND METHODS

Sampling the house finch population. At least once each week, we caught birds in wire mesh traps at bird feeders, measured, and released them as part of our ongoing monitoring and study of a house finch population. We collected data during the breeding season, from February 15, 1999, to August 15, 1999, although our estimates of individual birds' survival beyond the 1999 breeding season relied on our records of recapturing or resighting those birds through Summer 2000. We fitted each bird with a unique combination of three colored plastic leg bands and a numbered aluminum band. As part of our continuing study of the house finch breeding population on the Auburn University campus in east-central Alabama (32°36′7.2″ N; 85°29′5.6″ W), we monitored approximately 280 nest boxes at least every third day for nesting activity. Thus, we were able to observe the progressive laying of eggs and hatching of chicks.

Measuring infection. We made subjective measures of the clinical signs of mycoplasmal conjunctivitis using the following scale: 0 = eye appears entirely normal; 1 = minor swelling of the ring around the eye; 2 = moderate swelling around the eye, with lacrimal secretion present and possible eversion of the conjunctiva; and 3 = eye nearly hidden by severe swelling and possible crust-like buildup of secretions, eventually causing a mechanical blockage of vision. To determine incidence of MG infection in the nesting adults, we trapped the adults by placing modified Potter traps around their nest boxes when the chicks were 7–8 days old. We obtained blood samples from the adults at that time, from the chicks 3–4 days later (i.e., when the oldest nestlings were 11 days old), and from many other birds captured for routine banding purposes throughout the breeding season. We collected a small

amount of blood (~100–200 μl) by venipuncture of the brachial vein using a 26.5-gauge needle followed by collection of the blood with a heparinized microhematocrit tube. Plasma from each blood sample was screened for MG-specific antibodies using the serum plate agglutination assay (SPA) described by Luttrell et al. (15,16; InterVet, Inc., Millsboro, DE). The degree of agglutination of plasma samples was scored on a scale from 0 to 4, with a score of 2 or more considered a positive test for the presence of antibodies to MG. We swabbed the choana of all breeding adult birds and all nestlings for detection of the MG organism using the polymerase chain reaction (PCR) with MG-specific primers (11,12,22). We considered birds to be infected if they were positive by PCR, SPA, or clinical signs; although seroconversion does not necessarily indicate current infection, nearly all SPA+ birds in a separate study (8) were known to have cleared an infection only recently.

Assessing effects of MG infection on nestlings. We tested for effects of MG on nestling development by comparing infected chicks with an uninfected nestling in another nest that hatched within 1 wk of the infected chick. House finch chicks in this population show a slight age-related asynchrony in their development, with the oldest chicks within a nest remaining slightly larger than their younger siblings throughout the nestling period (authors' unpublished data). Therefore, we compared the development of each chick infected with MG to the development of an uninfected chick with the same place in the hatching order within its nest. For instance, an infected chick that was the first to hatch in its nest was compared with a first-hatched chick in another recently hatched nest. For each of these comparisons we made *a priori* predictions that MG infection would negatively impact development; thus, for these comparisons, we used one-tailed, paired *t*-tests.

Statistical analyses. We used Statview v. 4.1 (SAS Institute, Cary, NC) to conduct all statistical analyses. We used chi-squared analysis to test whether the occurrence of infections was distributed evenly among the breeding adults and those not known to be breeding. We used the same analysis to test for a nonrandom occurrence of disease according to hatch order among the nestlings and to test for any differences in the return rates of infected *vs.* uninfected chicks. To compare mean fecundity values between infected and uninfected females, we used the nonparametric Mann-Whitney *U*-test, and we used the nonparametric Wilcoxon signed ranks test for paired comparisons of infected chicks with comparable uninfected chicks.

Sampling effort and infection rates. We captured a total of 745 finches, including individuals from all age classes and both sexes, and have SPA and PCR data for a subset of them, including most of the breeding adults at all accessible nests on the Auburn University campus (Table 1). Our sample included 272 adults that we never observed at a nest; although many of those adults were possibly nesting outside our study site and were only captured because they visited our bird feeders, we refer to them as adults of unknown breeding status. Because some of the individuals known to be breeding re-nested and were sampled at more than one nest, we have a total of 73 samples taken from 57 breeding adults at 43 nests. However, to prevent pseudoreplication in our dataset, each individual was represented only once in any analysis. Any individual with a SPA-positive score was considered either to be currently infected or to have been infected, for the sake of analyses comparing infected *vs.* uninfected birds.

RESULTS

Effect of breeding status on infection rates. We found no differences between breeding adults and adults of unknown breeding status in incidence of clinical signs of mycoplasmal infection (chi-square test = 1.68; df = 1; $P = 0.20$) nor in prevalence of infection as measured by the SPA (chi-square test = 0.55; df = 1; $P = 0.46$) (Table 1). There was no significant difference in the prevalence of infection, as shown by the SPA, of known breeders and all birds of unknown status, including the hatch-year birds (chi-square test = 0.33; df = 1; $P = 0.57$).

Temporal pattern of infections in breeding adults. Individuals that tested positive by either SPA or PCR, or both, had more successful nests than did individuals with no evidence of infection (2.0 *vs.* 1.19 nests reaching the seventh day of brooding, respectively; $t = -2.43$; df = 8; $P = 0.04$, two-tailed). Other observations also suggest that re-nesting may increase the risk of infection; tests for the presence of MG among the breeding adults were typically negative early in the breeding season, whereas infections among breeding adults were detected relatively late in the breeding season. Furthermore, of 73 PCR tests for active MG infections, all 38 conducted from February to April were negative, and the nine infections we detected occurred during the second half of the breeding season, from May to August. The median sample date for those breeding adults found to be infected was May 29, 1999, as compared with April 12, 1999, for those samples that were negative both by SPA and PCR. By way of reference, the median sampling date for all nests combined was April 22, 1999, indicating that infections occurred in later breeding attempts. Possibly because of the late date

Table 1. Evidence of MG infection or exposure of house finches by age class.

Group	Clinical signs: No. pos./ No. examined (%)	SPA[A]-positive: No. pos./ No. tested (%)	PCR-positive: No. pos./ No. tested (%)
All adults	8/329 (2.4)	29/184 (15.8)	
Breeding adults	0/57 (0)	6[B]/57 (10.5)	6[B]/57 (10.5)
Adults of unknown breeding status	8/272 (2.9)	22/127 (17.3)	
Hatch-year birds	11/203 (5.4)	24/180 (13.3)	
Nestlings	0/213 (0)	0/213 (0)	10/213 (4.7)

[A]SPA = serum plate agglutination; PCR = polymerase chain reaction.
[B]These figures do not represent the same six individuals. Two birds tested positive only by SPA, two only by PCR, and an additional four were positive by both tests; eight breeding birds in total were infected with MG.

at which they became infected, only one of the infected adults re-nested after MG infection. That individual remained infected during its subsequent nesting attempt, and one of the five chicks in that subsequent nest became infected.

Effects on adult fecundity and return rates. We found no differences between infected females and uninfected females in the number of eggs laid, the hatchability of those eggs, or the number of chicks fledged (Table 2). We also found no differences between infected breeders and uninfected breeders in the probability of return the following year (chi-square test = 0.15; df = 1; $P = 0.70$). Three (38%) of eight infected breeding adults were resighted on the study site the next year, whereas 21 (43%) of 49 uninfected breeding adults were resighted the next year.

Frequency of transmission to nestlings. A total of 10 chicks from eight nests were PCR-positive for the presence of MG, although no chicks were SPA-positive. Six nests contained one infected chick each, and two additional nests contained two infected chicks each. In each of six nests where a breeding adult was known to be PCR-positive for MG, at least one of the chicks in the nest was also infected. However, in each of two nests where we were able to capture only one of the adults, which were PCR-negative, we found a PCR-positive chick. We assume that the uncaptured adults at those nests were PCR-positive but do not include those adults in analyses of effects of MG on breeding birds.

Effects of MG on nestling development. The distribution of infected chicks by hatch order was as follows: five infected chicks hatched first, two infected chicks hatched second, two infected chicks hatched third, and the remaining chick hatched fourth. The modal clutch size in house finches is five eggs (9); so, although this skew toward first-hatched chicks being infected is not significantly different from a random distribution (chi-square test = 7.0; df = 4; $P = 0.14$), it is suggestive of infection occurring among the oldest chicks. We found that infected nestlings had significantly smaller tarsi than did uninfected nestlings, but we detected no significant differences in the bill length, mass, or hematocrit levels of the two groups (Table 3).

Table 2. Mean (±SD) fecundity measures for female house finches infected with MG, compared with uninfected females.

	Infected females $n = 5$	Uninfected females $n = 23$	z	df	P
No. eggs laid	4.6 (0.55)	4.7 (0.63)	−0.12	26	0.91
No. eggs hatched	3.8 (0.84)	4.3 (0.81)	−0.72	26	0.47
No. chicks fledged	3.4 (1.14)	3.9 (1.32)	−0.81	26	0.42

Table 3. Mean variable (±SD) trait values of nestling house finches infected with MG, compared with uninfected nestlings.

	Infected nestlings $n = 10$	Uninfected nestlings $n = 10$	z	df	P
Tarsus length (mm)	15.8 (1.2)	16.4 (0.6)	−2.13	9	0.03
Bill length (mm)	5.0 (0.4)	5.1 (0.3)	0.01	9	0.99
Mass (g)	16.17 (2.2)	16.35 (1.6)	−0.36	9	0.72
Hematocrit (%; $n = 9$)	34.8 (5.0)	32.0 (7.7)	−0.89	9	0.37

Effects on recapture rates of nestlings. We found no difference between the rates at which we recaptured infected nestlings (0/10 = 0%) and uninfected nestlings (6/173 = 3.4%) the following year (chi-square test = 0.36; df = 1; $P = 0.55$), although this measure likely illustrates high dispersal rates for both groups rather than any differential survival to the following year (9). Significantly fewer uninfected nestlings (21/173 = 12%) were recaptured within 60 days of fledging than infected nestlings (4/10 = 40%; chi-square test = 6.19; df = 1; $P = 0.01$).

DISCUSSION

We found no difference in the prevalence of MG infection between birds known to be breeding and those of unknown breeding status and, unlike the situation reported by Hartup and Kollias (6) in a population of house finches in New York state, we found that nestlings as well as breeding adults were infected with MG. Contrary to our initial expectations, we found surprisingly little evidence that MG infection impacted the reproductive output of the breeding adults but did find suggestions that MG infection significantly impacted the development and dispersal of nestlings.

Our observation that parent house finches infect their offspring is consistent with Ley and Yoder's (13) report of both vertical and pseudovertical transmission of MG in poultry. However, we argue for pseudovertical rather than true vertical transmission because in two cases only the adult male at a nest was infected, and yet we still found infected chicks in those nests. Also, we failed to detect MG in chicks from one particular nest, even though the female attending the nest showed a strong SPA response. These observations, combined with the pattern of infection among the oldest chicks, suggest that infection occurs after hatching rather than transovarially. However, although we conclude that MG is being transmitted in a pseudovertical manner, we cannot exclude the possibility of vertical transmission until direct examination of female house finch follicles is accomplished.

Poultry infected with MG at subclinical levels show a depression in their rates of growth, hatchability, and egg production (13), so even low levels of MG infection could potentially impact the reproductive success of house finches in the wild. Furthermore, Faustino et al. (4) reported a decline in survivorship of birds seen to be infected with MG in a wild population of house finches in New York state. Our finding that birds raising the most chicks were more likely to be infected suggests that higher rates of reproduction carry a cost in the form of increased risk of disease. However, none of the breeding finches in our study showed clinical signs, and we were unable to detect any statistically significant impact of MG infection on the rates of hatchability or fledging or the rate at which we resighted breeding adults the next year. House finches in the eastern United States typically disperse after their first molt and then return annually to the site where they first attempt to breed, so a failure to resight an adult can reasonably be interpreted as mortality. This is especially true of the breeding adults in our study because they all bred successfully at the site in 1999 and therefore were likely to return in 2000 if they were alive (8). We did find that the nests of infected females showed lower rates of hatching and fledging, but these differences did not approach statistical significance.

The role of disease and parasitism in influencing reproductive fitness is potentially large, particularly if individuals are infected early in life. Chicks infected with even low levels of MG at a time when their bodies are undergoing rapid growth and development could potentially be permanently affected (18). For instance, the annual peak of MG infection on our study site occurs in September (21) when many juvenile birds are undergoing molt. Because MG infection decreases the brightness of plumage grown by male house finches during molt (2) and brighter plumage is preferred by female house finches in mate choice (7), even low-level MG infections of nestlings may lead to difficulties in attracting mates during the subsequent breeding season. Furthermore, decreased body size at the time of independence from parental care has been correlated with reduced over-winter survival and therefore reduced recruitment into the breeding population (19,24).

We observed just such an impact on body size among the infected chicks, which showed smaller tarsi than the uninfected chicks. However, house finches show extremely high dispersal rates for both sexes, with 95%–97% of chicks leaving their natal area soon after independence from their parents, making it difficult to estimate rates of survival or recruitment into the breeding population. Still, we were able to get at least a glimpse of any postfledging impacts of MG infection by observing the length of time the chicks remained on the study site until dispersal. Infected chicks were significantly more likely than uninfected chicks to be recaptured on the study site than were the uninfected chicks in the period up to 2 mo after

fledging. We see two possible explanations for this higher recapture rate, which are not mutually exclusive. First, the infected birds' dispersal may have been delayed by effects of the illness, with increased time on the study site increasing its probability of being recaptured. Alternately, infected birds may rely more than uninfected birds on easy access to food at our bird feeders; because we regularly trap at the feeders, an increased amount of time spent there by infected fledglings increases the likelihood of recapture. Either alternative implies a cost to the nestlings of being infected.

Still, our failure to find more pronounced effects of infection on nestlings, and even our finding of current infection in breeding adults, was somewhat surprising. We expected to see effects of infection on adult reproductive success, given the significant morbidity and mortality caused by the disease beginning in 1994 and continuing at least through 1997 (10). We suspect that the relationship between this bacteria and its host may be evolving, either through decreased virulence of MG, selection for resistance in the house finch, or a combination of the two.

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