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Source: Journal of Wildlife Diseases, 37(1) : 82-88

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-37.1.82

Journal of Wildlife Diseases, 37(1), 2001, pp. 82–88

CHARACTERIZATION OF THE MYCOPLASMAL CONJUNCTIVITIS EPIZOOTIC IN A HOUSE FINCH POPULATION IN THE SOUTHEASTERN USA

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ABSTRACT: An epidemiological study of the prevalence of mycoplasmal conjunctivitis in the house finch (*Carpodacus mexicanus*) was conducted in Auburn (Alabama, USA) between March 1998 and February 1999. Clinical disease was observed in 4% of the 1,214 finches trapped and examined. This rate is comparable to the average annual prevalence observed in this population since 1996, although the prevalence of clinical disease observed in the peak months of September through November was lower than in previous years. Clinically ill birds were observed in all months of the study. To estimate the prevalence of recovering and asymptomatic, infected birds, we tested a subset of 334 house finches serologically for exposure to *Mycoplasma gallisepticum* (MG) using the serum plate agglutination (SPA) assay. The prevalence of clinical disease in this subsample was slightly higher (7%) than in the entire sample, reflecting the fact that the serological survey was initiated in the late summer when the prevalence of MG infection peaks in our study population and a sampling bias for symptomatic birds. The serological survey indicated that 13% of this subpopulation had been exposed to MG. We also tested 46 of 334 finches by polymerase chain reaction (PCR) to detect MG in seropositive, asymptomatic birds. Use of the PCR in conjunction with the SPA detected six asymptomatic, infected birds that may represent potential carriers or birds in the early stages of infection. The decreasing prevalence of clinical disease observed during the peak months suggests a changing host-parasite relationship. Continued surveillance of this population, employing both clinical observation and serological analysis will be useful in characterizing these changes over time.

Key words: Behavioral ecology, *Carpodacus mexicanus,* epidemiology, house finch, *Mycoplasma gallisepticum,* mycoplasmosis, survey.

INTRODUCTION

In February 1994 conjunctivitis was first detected in house finches (*Carpodacus mexicanus*) observed at feeders in suburban Washington D.C. (USA) (Ley et al., 1996; Luttrell et al., 1996). By October 1994, the disease was reported from nine mid-Atlantic states and by November 1997 had been reported throughout the eastern range of the house finch (Dhondt et al., 1998), with several million house finches estimated to have died from the disease (Nolan et al., 1998). Mycoplasmas were isolated from affected house finches and subsequently identified as *Mycoplasma gallisepticum* (MG) by immunofluorescence (Ley et al., 1996; Luttrell et al., 1996). MG is an economically important pathogen of domestic poultry, causing acute respiratory disease and decreased feed conversion and egg production (Ley and Yoder, 1997). Prior to the occurrence of the disease in house finches, MG infections had been reported in wild turkeys (Fritz et al., 1992; Luttrell et al., 1991; Rocke et al., 1988), but MG infections in passerine birds had been reported infrequently with few individuals affected (Jain et al., 1971; Shimizu et al., 1979).

Juvenile house finches disperse long distances during the summer and fall (Hill, 1993) and it has been suggested that this behavior may be a factor in the rapid spread of MG throughout the eastern range of this species (Fischer et al., 1997). The social nature of the house finch and its utilization of artificial feeders in large numbers also have been implicated in the spread of mycoplasmal conjunctivitis (Hartup et al., 1998). To date, the American

goldfinch (*Carduelis tristis*) is the only other passerine showing significant susceptibility to this disease, and then only at levels much below those reported for the house finch (Ley et al., 1997).

The source of the MG strain infecting the house finch is unknown. Random amplification of polymorphic DNA (RAPD) analysis of the MG isolates obtained from house finches indicates that these isolates are distinct from those obtained from domestic poultry (Ley et al., 1997). RAPD analysis also indicates that a single MG strain is responsible for the epidemic, as geographically distinct isolates have virtually identical banding patterns (Ley et al., 1997).

The house finch population on the campus of Auburn University in east-central Alabama has been studied intensively since 1993. Conjunctivitis was first observed in several individuals in this population late in 1995. The prevalence of clinically ill birds in the Auburn (Alabama, USA) population peaked in late summer of 1996 when approximately 60% of the birds were symptomatic (Nolan et al., 1998). By November of that year, the prevalence of conjunctivitis in this population had decreased to 2 to 4%. A similar pattern of disease was observed the following year when conjunctivitis again peaked in September and October, although a smaller percentage of the birds (23%) were symptomatic in the peak months (Brawner, 2000).

The composition of the surviving population in late 1996 was significantly different than that prior to the epidemic (Nolan et al., 1998). The adult sex ratio of the surviving population was shifted from male-biased to female-biased and the mean body size of this population was significantly smaller.

Although it is difficult to estimate the mortality associated with mycoplasmal conjunctivitis in the wild, all untreated, symptomatic house finches housed in the Auburn University aviary during the summer of 1996 died. The east coast population appears to have suffered a serious decline, with infected birds presumably either dying from the illness or falling victim to starvation or predation because of the symptoms of the disease (Nolan et al., 1998; Sauer et al., 1997).

The well-characterized house finch population on the Auburn University campus provides a model system for the analysis of the impact of MG on the house finch and the changing nature of this host-parasite relationship. In this paper we present the results of an epidemiological survey of this population from March 1998 through February 1999 and describe an evolving relationship between the house finch and MG.

MATERIALS AND METHODS

Birds were captured as part of an on-going project on the behavioral ecology of the Auburn University campus (32°35′N, 85°28′W) house finch population. The birds were trapped using wire-mesh basket traps under permits from the Alabama Department of Conservation (Montgomery, Alabama, USA; No. 60) and the Bird Banding Laboratory (United States Geological Survey, Laurel, Maryland, USA; No. 21661). All procedures involving live animals were reviewed and approved by the Auburn University Institutional Animal Care and Use Committee (PRN #0111R2093). We took a series of standard morphological measurements and banded all birds. We made subjective measures of the degree of conjunctivitis on every bird using the following scale: 0 = eye appears entirely normal; 1 = minor swelling around the eye; 2 = moderate swelling around the eye, with eversion of the conjunctiva and lachrymal secretion present; 3 = severe swelling, with the eye nearly closed by swelling and/or crusting of the secretions.

Beginning in late July 1998, blood was collected from a subset of the birds to compare the estimate of the prevalence of MG infection provided by clinical data with the estimated prevalence provided by serological analysis. This subset was not a random sampling of the entire study population because the serological survey began in the late summer when the prevalence of conjunctivitis is peaking. In addition, the serological sampling was weighted towards symptomatic birds in an effort to provide a sufficiently large sample size for the comparison of SPA and PCR results. Age estimates were made for birds collected during the summer months when hatch-year birds can be

differentiated from adult birds. The birds were categorized as either hatch-year or adult by the plumage color and extent of plumage wear (Hill, 1993).

Blood for was collected by venipuncture of the brachial vein using a 26.5 gauge needle to collect approximately 100–200 μl of blood for serological analysis. To detect antibodies to MG we used a commercial serum plate agglutination (SPA) assay (Luttrell et al., 1996; Intervet Inc., Millsboro, Delaware). The extent of agglutination of plasma samples was scored on a scale from 0 to 4, with a score of ≥2 considered positive.

A smaller number of birds with and without clinical signs were selected for PCR evaluation. Samples for PCR analysis were obtained by gently swabbing the conjunctiva, choanal cleft and trachea of symptomatic house finches using a microtip swab (Becton Dickinson and Co., Sparks, Maryland, USA). To avoid damaging the healthy eye of asymptomatic birds, only the choanal cleft and trachea were swabbed in these birds. Individual swabs were suspended in 100 μl of sterile water. The samples were boiled for 10 min, incubated at −20 C for 10 min and centrifuged at 14,000 × g for 5 min. If not immediately used in the PCR assay, the specimens were stored at −20 C. MG DNA was amplified using two MG-specific primers (MG-14: 5′-GAG CTA ATC TGT AAA GTT GGT C-3′ and MG-13: 5′-GCT TCC TTG CGG TTA GCA AC-3′) which amplify a 185-base pair fragment from the 16S rRNA gene (Lauerman, 1998). The PCR amplification was performed as previously described (Lauerman, 1998; Lauerman et al., 1993, LTI, Gaithersburg, Maryland).

The prevalence of conjunctivitis in house finches in the summer of 1996 was compared to the prevalence of conjunctivitis in the summer of 1998 using the chi-square test. We also compared the prevalence of conjunctivitis in recaptured (resident) finches and newly captured (mostly migrant) finches using the chi-square test.

RESULTS

Of the 1,214 birds examined for clinical disease, 51 or 4% were symptomatic. Symptomatic birds were observed in every month of the study (Table 1). The prevalence of clinical disease varied monthly from 1 to 21%, peaking in the late summer and fall months of September and November (Table 1). The prevalence of clinical disease dropped in October, a month when we generally observe relatively few house finches at the feeders. Of the 51 symptomatic birds, 25 (49%) exhibited mild symptoms (MG score of 1), 13 (25%) had moderate lesions (MG score of 2) and 13 (25%) exhibited severe disease (MG score of 3).

TABLE 1. Monthly distribution of clinical conjunctivitis in house finches captured at Auburn University, 1998–99.

Month	Number with clinical disease	% of birds symptomatic
March 1998	4/62	7
April 1998	1/74	1
May 1998	1/95	1
June 1998	5/130	4
July 1998	5/222	2
August 1998	4/100	4
September 1998	8/39	21
October 1998	2/84	2
November 1998	7/71	10
December 1998	3/68	4
January 1999	5/108	5
February 1999	6/161	4

The SPA assay provided a higher estimate of MG infection than did observation of clinical disease in the 334 birds that were assessed serologically (Table 2). Twenty-four of these birds were symptomatic (7%), while 44 (13%) were seropositive by the SPA assay (Table 2). Of the 24 clinically ill birds, 21 (88%) were seropositive. The higher prevalence of clinical disease observed in this subset of the study population reflected the fact that this survey began late in the summer when the prevalence of clinical disease peaks and the sampling bias was toward symptomatic birds. Although the prevalence of clinical signs was higher in these birds than in the entire study population (7% versus 4%), the trends in the monthly distribution of the prevalence of clinical disease were similar to those seen in the larger population (Table 2).

Age data was available on 169 of the 334 birds included in the serological analysis. Of these birds, 11 of 157 hatch-year birds (7%) were symptomatic, while none of the

TABLE 2. Monthly distribution of clinical conjunctivitis and seroreactivity to *Mycoplasma gallisepticum* (MG) in a subset of house finches captured at Auburn University, 1998–99.

Time period	Number with clinical disease	% of birds symptomatic	Number MG−SPA[A] positive	% of birds SPA positive
July 1998	4/77	5	13	17
August 1998	2/61	3	7	12
September 1998	6/30	20	8	26
October 1998	0/8	0	1	13
November 1998	5/36	14	7	20
December 1998	0/18	0	1	6
January 1999	4/68	6	5	7
February 1999	3/36	8	2	6

[A] MG-SPA = MG Serum Plate Agglutination.

12 adult birds were symptomatic. Forty-five (29%) of hatch-year birds were SPA positive, while only one (8%) adult bird was seropositive.

We examined the use of PCR amplification of MG DNA to confirm the SPA results. We first tested the ability of MG-specific primers to amplify MG DNA directly from material collected with a swab (Fig. 1). These primers amplified a 185-base pair fragment in specimens obtained from symptomatic house finches (Fig. 1, lane 4). This fragment co-migrated with a DNA fragment amplified from a MG positive control specimen obtained from a MG-infected domestic chicken (Fig. 1, lane 2). No fragment was amplified in a specimen obtained from a seronegative, asymptomatic house finch or from a negative control specimen obtained from a domestic chicken (Fig. 1, lanes 3 and 5).

Samples from 27 seropositive and 18 seronegative birds were tested by PCR. MG DNA was detected in specimens from 23 or 85% of the SPA positive birds (Table 3). No DNA was amplified from any of the 15 asymptomatic, SPA negative birds tested. Of the 17 birds that had received a score of 2 on the SPA assay, MG DNA was detected by PCR in 13 (76%). When the clinical status of these 17 finches is considered, MG was detected in eight of eight (100%) of the symptomatic birds and five of nine (56%) of the asymptomatic birds (Table 3). The PCR assay also detected MG DNA in one asymptomatic, seropositive finch with a SPA score of 3. Thus, the combination of the SPA assay and PCR analysis detected an additional six infected birds that would have been missed if the birds were assessed on clinical disease alone. Addition of these six asymptomatic

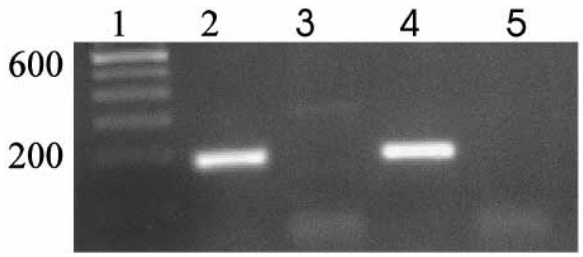

FIGURE 1. Amplification of *Mycoplasma gallisepticum* from the house finch using 16S rRNA primers. Lane 1 shows a 100 base-pair ladder; numbers on the left indicate fragment size. Lane 2 shows the positive control from MG infected chicken. Lane 3 shows the negative control from uninfected chicken. Lane 4: illustrates a specimen obtained from a symptomatic house finch. Lane 5 is from a specimen obtained from an asymptomatic house finch. The faint lower band observed in lanes 3 and 5 are unconsumed primers.

TABLE 3. Correlation of serologic and polymerase chain reaction (PCR) results for *Mycoplasma gallisepticum* (MG) in selected house finches from Auburn University in 1998–99.

	Number PCR positive (%)	
SPA[A] score	Asymptomatic	Symptomatic
0–1	0/15 (0)	3/3 (100)
2	5/9 (56)	8/8 (100)
3	1/1 (100)	6/6 (100)
4	—	3/3 (100)
Total	6/25 (24)	20/20 (100)

[A] SPA = Serum Plate Agglutination.

but infected finches to the 24 clinically ill birds provides an estimated prevalence of infection of 9% versus the 7% estimate based on clinical disease. While no MG DNA was detected by the PCR assay in any of the seronegative, asymptomatic finches, this assay did confirm MG infection in the three seronegative, symptomatic birds.

DISCUSSION

Our field analysis of the Auburn house finch population from March 1998 through February 1999 showed that MG persists in this population. Although the annual prevalence of conjunctivitis in our population was approximately 3 to 5%, the prevalence of clinical disease observed during the peak period in late summer and early fall has declined significantly from a peak of 60% observed in the summer months of August and September of 1996 to 9% in these same months in 1998 ($\chi^2 = 79.68$, $P = 0.0001$).

Our results reveal an epidemic that is distinct from that reported in the epidemic in the northeast (Dhondt et al., 1998; Hartup et al., 1998, 2000). In our study, symptomatic birds were observed throughout the year at a low, but relatively steady frequency, although our results identify a peak in both infection and disease in the late summer and early fall in Alabama. In contrast, in the northeast, clinical disease is most prevalent in the months from November to March (Hartup et al., 1998, 2000). Our analysis of age-related disease frequency suggests that hatch-year birds provide a susceptible population in the late summer and early fall and infection in this population may be responsible for the peak of clinical disease and seroresponsiveness we observed at this time of the year. Hartup and Kollias (1999) have demonstrated that the risk of MG infection for nestlings is very small in the northeast. If this finding holds true for the southeast as well, hatch-year birds may be at greatest risk of infection when they join large flocks of juvenile birds and congregate at feeders (Hill, 1993). In the northeast, with cold winters and mild summers, cold periods in the winter present the greatest weather-related stress to house finches. In our southeastern study area, the winters are typically mild, and the very hot days of mid- and late summer present the greatest weather stress to house finches.

A possible alternative explanation for the low incidence of MG that we observed in the winter is that our winter sample included migrant birds from the north (Able and Belthoff, 1998; Belthoff and Gauthreaux, 1991) and that this migratory population included only disease-free, healthy individuals. We have tested this idea by comparing the prevalence of conjunctivitis in newly banded birds, assumed to include mostly migrants, and previously banded birds, recaptured from the previous summer and hence known to be residents during December through April when migrants are present. No difference in the prevalence of conjunctivitis in these two groups was found ($\chi^2 = 0.009$, $P = 0.86$).

Not unexpectedly, we obtained a higher estimate of the prevalence of exposure to MG in the population from the serological survey than from the clinical observation data. Previous studies have demonstrated that the SPA and PCR assays are useful in confirming clinical findings (Fischer et al, 1997; Hartup and Kollias, 1999; Hartup et al., 2000; Luttrell et al., 1996). Rapid, inexpensive serological assays are extremely useful in large-scale epidemiological stud-

ies and provide a potential means of identifying birds that are infected but asymptomatic as well as recovering birds. Although the SPA assay is rapid and relatively inexpensive, nonspecific reactions have been reported in poultry and there is no data on the occurrence of false positive reactions in house finches (Ley and Yoder, 1997; Luttrell et al., 1996). Our results demonstrate that confirmation of positive serological results by PCR permits the detection of asymptomatic, infected birds and provides a more complete estimate of the prevalence of MG infection. As this relatively new host-parasite relationship evolves, the ability to identify these asymptomatic, infected individuals may become more important. Continued analysis of this relationship will allow us to determine whether a stable interaction between host and parasite will develop.

The results were report in this paper also have implications for the behavioral ecology of the host species. Male house finches with red ornamental plumage are preferred as mates more often than are duller, orange or yellow males (Hill, 1990, 1991, 1994). Mycoplasmosis impairs expression of ornamental plumage by male house finches (Brawner et al., 2000; Nolan et al., 1998), so males infected with MG while undergoing their annual molt will develop a dull plumage that will decrease their likelihood of being chosen as a mate. Furthermore, the annual peak prevalence of infection we report here coincides with the period when males are most likely to be molting into their new plumage. Therefore, if MG becomes an established parasite of this host, its effects on the host's health may help to maintain the honesty of red plumage as a signal of male condition.

ACKNOWLEDGMENTS

We thank the members of the Hill laboratory including K. McGraw, A. Stoehr, R. Duckworth, A. Dervan and the Roberts laboratory, K. Klenk, C. Roland, J. Welden and A. Dreibelbis for help with the capture of birds and serology. We also thank F. Hoerr, S. Rowe and D. Kiser at the C. S. Roberts Veterinary Diagnostic Laboratory and P. Luttrell at the Southeastern Cooperative Wildlife Disease Study for advice on the serological methodologies. Support for this work was provided by the National Science Foundation (Grant #IBN9722171 to G.E.H), an Undergraduate Research Award from the Department of Biological Sciences, Auburn University, the Walter F. Coxe Research Fund of the Birmingham Audubon Society, the College of Science and Mathematics at Auburn University, the Alabama Agricultural Experiment Station, and the Department of Biological Sciences at Auburn University.

LITERATURE CITED

ABLE, K. P., AND J. R. BELTHOFF. 1998. Rapid 'evolution' of migratory behaviour in the introduced house finch of eastern North America. Proceedings of the Royal Society of London—Series B: Biological Sciences. 265: 2063–2071.

BELTHOFF, J. R., AND S. A. J. GAUTHREAUX. 1991. Partial migration and differential winter distribution of house finches in the eastern USA. Condor 93: 374–382.

BRAWNER, W. R. III., G. E. HILL, AND C. A. SUNDERMANN. 2000. Effects of coccidial and mycoplasmal infection on carotenoid-based plumage coloration in male house finches. Auk. 117: In press.

DHONDT, A. A., D. L. TESSAGLIA, AND R. L. SLOTHOWER. 1998. Epidemic mycoplasmal conjunctivitis in house finches from eastern North America. Journal of Wildlife Diseases 34: 265–280.

FISCHER, J. R., D. E. STALLKNECHT, M. P. LUTTRELL, A. A. DHONDT, AND K. A. CONVERSE. 1997. Mycoplasmal conjunctivitis in wild songbirds: the spread of a new contagious disease in a mobile host population. Emerging Infectious Diseases 3: 69–72.

FRITZ, B. A., C. B. THOMAS, AND T. M. YUILL. 1992. Serological and microbial survey of *Mycoplasma gallisepticum* in wild turkeys (*Meleagris gallopavo*) from six western states. Journal of Wildlife Diseases 28: 10–20.

HARTUP, B. K., AND G. V. KOLLIAS. 1999. Field investigation of *Mycoplasma gallisepticum* infections in house finch (*Carpodacus mexicanus*) eggs and nestlings. Avian Diseases: 43: 572–576.

———, ———, AND D. H. LEY. 2000. *Mycoplasmal* conjunctivitis in songbirds in New York. Journal of Wildlife Diseases 36: 257–264.

———, H. O. MOHAMMED, G. V. KOLLIAS, AND A. A. DHONDT. 1998. Risk factors associated with mycoplasmal conjunctivitis in house finches. Journal of Wildlife Diseases 34: 281–288.

HILL, G. E. 1990. Female house finches prefer col-

orful mates: sexual selection for a condition-dependent trait. Animal Behaviour 40: 563–572.

———. 1991. Plumage color is a sexually selected indicator of male quality. Nature 350: 337–339.

———. 1993. House finch (*Carpodacus mexicanus*). *In* The birds of North America, A. Poole, and F. Gill, (eds.). The American Ornithologist's Union, Washington, DC, pp. 1–24.

———. 1994. Geographic variation in male ornamentation and female mate preference in the house finch: a comparative test of models of sexual selection. Behavioral Ecology 5: 64–73.

JAIN, N. C., N. K. CHANDIRAMANI, AND I. P. SINGH. 1971. Studies on avian pleuro-pneumonia-like organisms. 2. Occurrence of *Mycoplasma* in wild birds. Indian Journal of Animal Science 41: 301–305.

LAUERMAN, L. H. 1998. Mycoplasma PCR Assays. *In* Nucleic acid amplification assays for diagnosis of animal diseases, L. H. Lauerman (ed.). American Association of Veterinary Laboratory Diagnosticians, Turlock, California, pp. 41–43.

———, F. J. HOERR, A. R. SHARPTON, S. M. SHAH, AND V. L. VAN SANTEN. 1993. Development and application of a polymerase chain reaction assay for *Mycoplasma synoviae.* Avian Diseases 37: 829–834.

LEY, D. H., J. E. BERKHOFF, AND S. LEVISOHN. 1997. Molecular epidemiological investigations of *Mycoplasma gallisepticum* conjunctivitis in songbirds by random amplified polymorphic DNA analyses. Emerging Infectious Diseases 3: 375–380.

———, ———, AND J. M. MCLAREN. 1996. *Mycoplasma gallisepticum* isolated from house finches (*Carpodacus mexicanus*) with conjunctivitis. Avian Diseases 40: 480–483.

———, AND H. W. YODER, JR. 1997. *Mycoplasma gallisepticum* infection. *In* Diseases of poultry, 10th Edition, B. W. Calnek, H. J. Barnes, C. W. Beard, L. R. McDougald, and Y. M. Siaf (eds.). Iowa State University Press, Ames, Iowa, pp. 194–207.

LUTTRELL, M. P., J. R. FISCHER, D. E. STALLKNECHT, AND S. H. KLEVEN. 1996. Field investigation of *Mycoplasma gallisepticum* infections in house finches (*Carpodacus mexicanus*) from Maryland and Georgia. Avian Diseases 40: 335–341.

———, S. H. KLEVEN, AND W. R. DAVIDSON. 1991. An investigation of the persistence of *Mycoplasma gallisepticum* in an eastern population of wild turkeys. Journal of Wildlife Diseases 27: 74–80.

NOLAN, P. M., G. E. HILL, AND A. M. STOEHR. 1998. Sex, size and plumage redness predict house finch survival in an epidemic. Proceedings of the Royal Society of London Series B: Biological Sciences 265: 961–965.

ROCKE, T. E., T. M. YUILL, AND T. E. AMUNDSON. 1988. Experimental *Mycoplasma gallisepticum* infections in captive-reared wild turkeys. Journal of Wildlife Diseases 24: 528–532.

SAUER, J. R., HINES, J. E., G. GOUGH, I. THOMAS, AND B. G. PETERJOHN. 1997. The North American Breeding Bird Survey Results and Analysis. Patuxent Wildlife Research Center, Laurel, Maryland. Version 96.4.

SHIMIZU, T., K. NUMANO, AND K. UCHIDA. 1979. Isolation and identification of mycoplasmas from various birds: an ecological study. Japanese Journal of Veterinary Science 41: 273–282.

Received for publication 8 January 2000