DYNAMICS OF CONJUNCTIVITIS AND MYCOPLASMA GALLISEPTICUM INFECTIONS IN HOUSE FINCHES

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ABSTRACT.—Conjunctivitis, an infectious disease caused by Mycoplasma gallisepticum (MG), has produced a significant decline in eastern House Finches (Carpodacus mexicanus) of North America. In this paper, we present findings from two complementary studies designed to clarify annual and seasonal trends of MG infections in House Finches from the northeastern United States. The first was a field study of House Finches common to urban and residential habitat from Mercer County, New Jersey. We documented conjunctivitis in 11% (188/1,651) of the birds examined. Conjunctivitis prevalence in House Finches ranged from 0 to 43% per month, and exhibited marked seasonal fluctuation (elevations during fall and winter months and lower disease prevalence during the breeding season). There was excellent intermethod agreement on disease prevalence when measured by either presence of physical signs (conjunctivitis) or MG infection (kappa = 0.75). During the peak of the breeding season (April through June), conjunctivitis was present in a greater proportion of males lacking a cloacal protuberance than males with a cloacal protuberance (P < 0.01), but was similar between breeding and nonbreeding females. The second study, a volunteer survey, revealed the proportion of northeastern U.S. monitoring sites with at least one diseased House Finch each month ranged from a peak of 59% (August 1995) to a minimum of 12% (July 1999). Subsequent to the epidemic peak of disease in 1995, a series of recurring cycles occurred, with elevations in those proportions noted in late fall and winter and minima during the breeding season. Mycoplasmal conjunctivitis now appears endemic among House Finches of that region and demonstrates dynamics consistent with annual variation in host density. Received 17 December 1999, accepted 5 September 2000.

MYCOPLASMAL CONJUNCTIVITIS is a recently described infectious disease of House Finches (Carpodacus mexicanus) in eastern North America (Ley et al. 1996, Luttrell et al. 1996, Fischer et al. 1997). The ocular lesions are the result of inflammatory sequelae to infection with Mycoplasma gallisepticum (MG), a common respiratory pathogen of domestic poultry (Jordan 1996, Ley and Yoder 1997). Several investigators have described various facets of the epidemiology of this emergent disease and the potential for spread of the disease to other avian hosts (Ley et al. 1997, Dhondt et al. 1998, Hartup et al. 1998, 2000, 2001; Luttrell et al. 1998, Stallknecht et al. 1998). The arrival of mycoplasmal conjunctivitis is correlated with significant population declines across much of the range of the eastern House Finch (Hochachka and Dhondt 2000), representing the estimated loss of tens of millions of individuals (Nolan et al. 1998). Despite the broad geographic scale of that disease's impact on its host, few estimates of disease prevalence in free-ranging populations have been proposed (Hartup et al. 2000), and no studies have investigated the association of disease with individuals of differing sex or age class, or physical characteristics in both wintering and breeding populations. Those observations are invaluable for assessing the potential of mycoplasmal conjunctivitis to limit House Finch populations.

The current understanding of the spatial and temporal dynamics of mycoplasmal conjunctivitis in free-ranging House Finch populations has come from an untraditional source: the House Finch Disease Survey (HFDS; Dhondt et al. 1998). That survey has documented the rap-
id spread of conjunctivitis in eastern House Finches via a network of volunteer observers (Fischer et al. 1997) and helped to elucidate potential risk factors for conjunctivitis in northeastern finches that utilize bird feeders (Hartup et al. 1998). The survey produces a monthly estimate of disease frequency that equals the proportion of monitored sites in a specified region where at least one diseased House Finch was observed. The HFDS provides a valid index to the prevalence of disease in House Finch populations determined by traditional field methods (Dhondt et al. 1998) because the presence of conjunctivitis in individual finches is closely correlated with active MG infections (Hartup et al. 2000).

The primary objective of this study was to document the occurrence of conjunctivitis and MG infections in House Finches common to New Jersey residential feeding stations for several years, and to contrast those findings with HFDS disease frequency estimates from the northeastern United States for the same period. In addition, we retrospectively analyzed banding records to ascertain the association of various demographic and morphologic characteristics with mycoplasmal conjunctivitis at different times of year.

**METHODS**

Field study.—House Finches were captured in Mercer County, New Jersey during 46 of 60 months between November 1994 and October 1999. Birds were captured at two residential sites in the city of Trenton, New Jersey, with Potter traps (Bub 1991) under valid state and federal permits at a minimum frequency of two weekends per month. All birds were banded with unique numbered aluminum leg bands and given a physical examination that included close inspection of the eyes and adnexa for signs of conjunctivitis, such as eyelid or conjunctival swelling, erythema, and discharge. The age of each bird was determined through plumage characteristics and extent of skull ossification (Pyle 1997), and classified as either a juvenile/hatch-year (HY) or an after hatch-year adult (AHY). Wing chord length (millimeters), weight (grams), and a furcular fat score (0 to 5 scale, 0 = no visible fat deposits, 5 = extensive fat deposits) were also determined. Females in breeding condition were determined by the presence of one of the following between March and August: a brood patch, palpable egg, or cloacal protuberance. The presence or absence of a cloacal protuberance was noted in males during the same period.

Between February 1998 and October 1999, diagnostic conjunctival swab samples were obtained from 586 House Finches for MG culture and polymerase chain reaction (PCR) testing. Samples were collected consecutively from all individuals captured during biweekly trapping sessions (some supplementary banding without sampling occurred during off weeks). Conjunctival swabs taken in the field were immediately immersed in mycoplasma broth and held under refrigeration for 24–48 h. Samples were then shipped by overnight mail to the laboratory and incubated 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 were tested for the presence of MG-specific DNA by PCR (Lauerman 1998). Twenty MG isolates made during the study were later compared by random amplification of polymorphic DNA fingerprinting (RAPD) using two different primer sets (Geary et al. 1994, Fan et al. 1995). RAPD assays included DNA extracts from a historical House Finch MG isolate and a MG vaccine strain used in commercial poultry (F strain) for comparison.

Disease frequency data are presented as either the proportion of individuals with conjunctivitis or a MG infection (culture or PCR positive) among individuals sampled each month during the study. Wing chord lengths, body weight, and fat scores of House Finches with conjunctivitis were compared to those of healthy House Finches using logistic regression (Hosmer and Lemeshow 1989). Sex and season of capture (March through August vs. September through February) were included as potential confounding variables in each analysis (StatView 5 statistical software, 1998, SAS Institute Inc., Cary, North Carolina, USA). Potential associations between sex, age, season, breeding condition, and conjunctivitis were evaluated with Mantel-Haenszel chi-square tests or Fisher’s exact test (EpiInfo v. 6.04, 1997 version, Centers for Disease Control and Prevention, Atlanta, Georgia, USA). The association between breeding condition and conjunctivitis in House Finches was assessed by using only observations made during the peak breeding months of April through June, when nearly all AHY House Finches were expected to be in breeding condition (Hill 1993). Monthly disease prevalence among HY House Finches was analyzed for linear trends using chi-square methods prior to October when aging HY House Finches becomes problematic (Schlesselman 1982, Pyle 1997). Statistical significance for all tests was established at $P < 0.05$. The significance of agreement between the two measures of disease in the study population (conjunctivitis and MG infections confirmed through laboratory analysis) was assessed by calculating a kappa test statistic (Martin et
al. 1987). Significant agreement was indicated with kappa > 0.70.

Volunteer survey.—The methodology of the HFDS has been thoroughly described elsewhere (Dhondt et al. 1998, Hartup et al. 1998). Briefly, the survey was originally designed to follow the spread of conjunctivitis in eastern House Finches by acquiring year-round observations from experienced volunteers via a questionnaire. Daily observations of healthy and conjunctivitis-affected House Finches were collapsed into one of two cumulative monthly categories: "healthy" for sites with daily observations of normal appearing birds only, and "diseased" for sites with at least one daily observation of a bird with conjunctivitis. We minimized potential observer error (misdiagnosis, including injury or other infectious disease) by screening each data form and excluding observations based on available descriptions and consultation with two avian disease specialists. The monthly reports were considered independent observations of a dynamic study population of birds, and not as repeated measures. We used 10,140 monthly HFDS observations made by 1,747 participants from eight northeastern states (Delaware, Maryland, New Jersey, Pennsylvania, New York, Connecticut, Rhode Island, and Massachusetts) to detect conjunctivitis in House Finches between November 1994 and October 1999. Data from the sites were used to determine the monthly proportion of sites with conjunctivitis-affected House Finches during the five-year period. A locally weighted regression technique was used to smooth the bivariate scatterplot and identify seasonal fluctuations among the monthly HFDS data (LOWESS; Cleveland 1981). Simple linear regression was used to assess the overall trend in disease frequency since the inception of the HFDS (Sokal and Rohlf 1980).

RESULTS

Conjunctivitis was observed in 11% (n = 188) of 1,651 House Finches examined from Mercer County, and occurred in 0 to 43% of House Finches when stratified by month (Fig. 1). Seasonal fluctuation in conjunctivitis prevalence was observed in the study population. Annual peak disease prevalence occurred during fall or winter, and lower disease prevalence in the breeding season, though there was limited follow-up during midsummer for several years. Proximate to the month of May, conjunctivitis prevalence either declined dramatically or the disease was not observed following several months at detectable levels. Banding records showed that the proportion of diseased AHY House Finches was greater between September and February (16%, n = 534) than during the breeding season of March through August (5%, n = 123; χ² = 10.5, P < 0.01). The proportion of diseased males and females, however, was similar within each season. During the peak of the breeding season, disease was most prevalent in males without a cloacal protuberance (24%, n = 25) compared to males with a cloacal protuberance (5%, n = 100; Fisher’s exact test P < 0.01). Males without a cloacal protuberance were nearly six times as likely to be diseased than males with a cloacal protuberance between the months of April and June, whereas disease prevalence was similar between breeding and nonbreeding females during this time.
FIG. 2. Monthly prevalence of conjunctivitis and MG infections among Mercer County, New Jersey House Finches 1998–1999. No birds were captured during April 1998 (NS), and no diagnostic samples were available in May 1998 (asterisk). Error bars denote the 95% confidence limit of the monthly estimates.

A seasonal change in disease prevalence was also observed in HY House Finches. The proportion of diseased HY House Finches exhibited a linear increase over June (2%, n = 54), July (2%, n = 167), August (9%, n = 95) and September (14%, n = 101; $\chi^2 = 15.0$, df = 1, $P < 0.01$). Hatch-year House Finches were more than eight times as likely to be observed with conjunctivitis in September than in June. The prevalence of conjunctivitis in HY and AHY House Finches was similar during that period.

Each of the remaining host characteristics we measured were not statistically associated with presence of conjunctivitis in House Finches. Among AHY House Finches, fat scores, weight, and wing chord lengths were not significantly different between diseased and nondiseased individuals when controlled for sex and season of observation.

Of the 586 birds sampled, M. gallisepticum was cultured from 51 House Finches and 10 culture-negative House Finches were positive for MG by PCR during 19 months of monitoring. The monthly prevalence of MG infections ranged from 0 to 44% during this period (Fig. 2). Clinical conjunctivitis and MG infection prevalence exhibited similar seasonal change; both were at a low level during the two monitored breeding seasons. An unexpected decrease in disease prevalence was observed in December 1998 for unknown reasons. There was agreement between an individual’s clinical status and laboratory findings in 95% (556/586) of birds sampled, indicating excellent intermethod concordance for identification of MG-associated conjunctivitis in House Finches (kappa = 0.75). The remaining variability between diagnostic tests (reflected in the monthly prevalence estimates in Fig. 2) is likely due to the presence of newly infected birds without clinical disease, MG carriers without conjunctivitis, or birds that have cleared the MG infection but not resolved the inflammatory lesions.

DNA fingerprints of 20 House Finch MG isolates showed no apparent differences in RAPD banding patterns over this same period (Fig. 3). The DNA profiles were also similar to a 1994 House Finch-derived MG isolate from North Carolina, but were different from a common MG vaccine strain. These relationships were confirmed by using a second set of primers in the RAPD assay (Fan et al. 1995; data not shown), and suggest the persistence of a single MG strain in the study population.

The proportion of northeastern U.S. monitoring sites with at least one diseased House Finch each month ranged from 59% in August 1995, to a minimum of 12% in July 1999 (Fig. 4). There was an initial increase in the proportion of sites reporting diseased House Finches in the first year of the survey despite marked month-to-month fluctuation. Subsequent to the peak of disease in 1995, a series of recurring cycles occurred, with elevations in the survey’s monthly proportions noted in late fall and winter and minima during the breeding season, often proximate to the month of April or May. Overall, proportion of northeastern sites reporting
DISCUSSION

The results of this study revealed repeated seasonal fluctuations of MG-associated conjunctivitis among northeastern House Finches. Conjunctivitis occurred in both sexes and all age classes of House Finches with similar frequency, and most commonly during the fall and winter months, coinciding with influx of large numbers of dispersing juveniles, migratory movements and the formation of winter flocks (Belthoff and Gauthreaux 1991, Hill 1993, Able and Belthoff 1998). Those factors are likely to increase the probability of MG transmission among House Finches as host density and social interaction reach their highest levels during those seasons (May and Anderson 1979, Hill 1993), but may also influence dramatic month-to-month variation in local disease frequency, as was observed in December 1998. Though conjunctivitis was rare in breeding House Finches and may have become locally extinct in Mercer County during mid-1995 and 1997, we were able to confirm the persistence of the disease among breeding House Finches and their offspring in 1998 and 1999 with more consistent sampling. This population apparently has yet to reach a threshold density below which MG infections are driven to extinction (Onstad 1993).

Seasonal fluctuation in the frequency of conjunctivitis in northeastern House Finches was suggested as early as 1996 from HFDS data (Dhondt et al. 1998, Hartup et al. 1998), but long-term confirmation of the underlying mycoplasmal infections was lacking. Our data show there is significant concordance of disease estimates on the basis of visual examination of the eyes and standard laboratory testing, and that a single pathogenic strain of MG is circulating in House Finches with no detected evidence of genetic variation or altered virulence (Ley et al. 1997, Hartup et al. 2000). Though an imperfect test (there are several other known causes of conjunctivitis in birds, Williams 1997), we are confident that the observation of conjunctivitis in individual House Finches correlates with the presence of the "House Finch strain" of MG in local populations. Thus, the HFDS properly provides a valid index to mycoplasmal disease patterns over larger geographic areas, but lacks resolution to discern short-term variation in local populations. Based on five years of monitoring, the HFDS shows mycoplasmal conjunctivitis is now at an endemic level, but may be in decline, among northeastern House Finches subsequent to the epidemic phase of the outbreak in 1995.

Studies of captive (Luttrell et al. 1998) and wild House Finches (Nolan et al. 1998), along with nationwide abundance indices (Hochachka and Dhondt 2000) suggest that mycoplasmal conjunctivitis has a profound effect on host survival and is linked with substantial population declines in eastern House Finches. Gross differences in survival probabilities of diseased and normal finches have not been detected in our study population to date (data not shown). In fact, we documented several instances of individual House Finches surviving MG infections and resolving their clinical disease in the current study sample. Together with the HFDS trends described above, we believe an increasingly greater proportion of the host population may now be resistant to MG compared to several years ago.

We observed a significant association between the lack of cloacal protuberance and mycoplasmal conjunctivitis in male House Finches during the peak breeding season in northern
New Jersey. That finding suggests that the disease is significantly more common in individual males of nonbreeding condition (as determined indirectly by lack of cloacal protuberance), though a causal link cannot be established from this information. Mycoplasmal infection may alter normal seasonal endocrine profiles that limit or delay the achievement of breeding condition, or male House Finches that do not achieve breeding condition for other reasons may be more susceptible to the disease. Further study is required to ascertain the disease status of male House Finches of known breeding condition to quantify the potential negative influence of mycoplasmal conjunctivitis on male reproductive performance.

House Finches exhibiting traits consistent with breeding, however, were not entirely free of disease. In both sexes, a small number of MG infections were detected. In MG infected House Finches, there is likely a trade-off between current and future reproductive effort and development of an immune response (Gustafsson et al. 1994). Constraints on breeding condition or productivity caused by MG infections would represent another, albeit minor, factor contributing to population limitation in eastern House Finches combined with direct mortality due to disease. MG infection in breeding poultry often negatively influences productivity through reduction in egg production (Nunoya et al. 1997) or lowered growth rates of offspring (Ley and Yoder 1997). At present, however, there is little to no evidence from field or laboratory studies to suggest that fecundity or productivity have been negatively influenced in House Finches by MG infections. More importantly, MG infections in breeding adults likely represent a significant risk for transmission of disease to immunologically naïve offspring and hence the perpetuation of the disease in local populations through the breeding season (Hartup and Kollias 1999), as well as spread of MG to disease-free regions by dispersing juveniles (Dhonadt et al. 1998). Carefully executed field and laboratory studies are needed to clearly demonstrate the causal associations between MG infection, mate choice, and reproductive outcomes to more definitively ascertain the effects of the disease on House Finch population dynamics.

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