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RISK FACTORS ASSOCIATED WITH MYCOPLASMAL CONJUNCTIVITIS IN HOUSE FINCHES

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ABSTRACT: Observations from a citizen-based survey were used to identify potential risk factors associated with mycoplasmal conjunctivitis (*Mycoplasma gallisepticum*) in eastern house finches (*Carpodacus mexicanus*). Between November 1994 and October 1996, 778 volunteers provided 7,224 monthly observations at residential bird feeding sites across an eight state region in the eastern USA. Information collected by questionnaires included health status of house finches and four sympatric passerine species, types and number of bird feeders maintained, neighborhood housing locale and altitude of the observation site. Bivariate analyses revealed that house finches were 14 to 72 times as likely to be observed with conjunctivitis than the sympatric species studied. Year of the study, season, and the presence of platform, hopper, and tube type feeders were significantly associated with conjunctivitis in house finches. Multivariate analysis using a logistic regression model suggests that increased risk of conjunctivitis in house finches was associated with the second year of the study (the third year of the outbreak), the cooler non-breeding periods from September through March, and the presence of tube style feeders. In addition, the presence of raised platform type feeders may have been protective against conjunctivitis in house finches. Prevention of spread of this disease may include modifying bird feeding activities based on season and type of feeder.

Key words: *Carpodacus mexicanus*, citizen science, conjunctivitis, epidemiology, house finches, *Mycoplasma gallisepticum*, risk factors, survey.

INTRODUCTION

An epidemic of conjunctivitis in house finches (*Carpodacus mexicanus*) was first recognized in February 1994 in the eastern USA (Fischer et al., 1997). Signs in affected finches included swollen or crusty eyelids, debilitation and a propensity to remain on or around bird feeders. Subsequent field and laboratory investigations resulted in repeated isolations of *Mycoplasma gallisepticum* (MG) from affected birds across a broad geographic area (Ley et al., 1996; Luttrell et al., 1996). Transmission studies later confirmed MG as the causative agent of conjunctivitis in eastern house finches (Fischer et al., 1997).

MG is frequently associated with respiratory tract disease, debilitation and carcass condemnation, and reduced egg production in domestic poultry (Mohammed et al., 1987; Yoder, 1991; Jordan, 1996). Cases of keratoconjunctivitis attributed to MG have rarely been reported in chickens (Nunoya et al., 1995). Experimental trials have shown that exposure to the house finch strain of MG elicits seroconversion in chickens, but not clinical disease (D. Stallknecht, pers. commun.).

Historically, MG has not been considered a naturally occurring pathogen in wild birds, although isolations have been documented from Japanese tree sparrows (*Passer montanus*), house sparrows (*Passer domesticus*), wild turkeys (*Meleagris gallopavo*), and captive-reared chukar partridges (*Alectoris chukar*) and ring-necked pheasants (*Phasianus colchicus*) (Jain et al., 1971; Shimizu, et al., 1979; Fritz et al., 1992; Cookson and Shivaprasad, 1994). Serologic surveys and experimental infections have suggested house sparrows may act as transient carriers of MG (Stallknecht et al., 1982; Kleven and Fletcher, 1983), but sustained reservoirs in passerines have not been clearly identified.

The house finch strain of MG has since

been isolated from eastern American goldfinches (*Carduelis tristis*) at numerous sites, a downy woodpecker (*Picoides pubescens*) from Michigan, and a blue jay (*Cyanocitta cristata*) housed in a rehabilitation setting where infected house finches were earlier maintained (Fischer et al., 1997). A similar clinical syndrome was observed in purple finches (*Carpodacus purpureus*) from Virginia (Porter, 1994). Molecular epidemiological studies have linked the outbreak in house finches and goldfinches (Ley et al., 1997).

In domestic poultry, MG may be transmitted by direct contact, air-borne dust or droplets, contact with contaminated surfaces, and through eggs to developing embryos (Yoder, 1991). The modes of transmission of MG in house finches are unknown, but are presumed to be enhanced by social and foraging behavior at bird feeders (Fisher et al., 1997). Identification of the factors involved in transmission and infection, such as season and bird feeder types, may play a significant role in understanding the spread of MG to susceptible hosts and in further investigations of this disease.

In November 1994, the Cornell Laboratory of Ornithology initiated the House Finch Disease Survey (HFDS). The objective of the HFDS was to track the spread of conjunctivitis (presumably MG-associated) throughout the eastern range of the house finch by using an established network of volunteers from Project FeederWatch, a large citizen-based bird feeder monitoring program. The HFDS has been successful in documenting the rapid spread of the epizootic throughout the range of the eastern house finch population and to American goldfinches based on respondents' comments from the questionnaire (Fischer et al., 1997).

Our objective was to identify potential risk factors associated with conjunctivitis in house finches at residential bird feeding stations using observations from the HFDS.

MATERIALS AND METHODS

Observations of house finches, purple finches, black-capped chickadees (*Parus atricapillus*), house sparrows, and dark-eyed juncos (*Junco hyemalis*) from eight states (Connecticut, Delaware, Maryland, Massachusetts, New Jersey, New York, Rhode Island and Pennsylvania) of the United States were made by volunteers between November 1994 and October 1996 (Fig. 1). This region was chosen for study because mycoplasmal conjunctivitis in house finches had been confirmed over much of this area by November 1994, initial HFDS data from these states suggested similar prevalence of disease, and the region provided an adequate sample size of observations for statistical analysis.

The 778 volunteers were recruited non-randomly from an initial mailing to over 9,000 experienced Project FeederWatch participants in the eastern USA and Canada. Respondents were provided a packet with detailed instructions, including a description of easily observed clinical signs consistent with conjunctivitis in house finches (reddened, swollen eyes, possibly with moist discharge). The use of this observer network allowed for immediate and economical collection of data across a large geographic area by experienced and motivated volunteers.

The data were collected using a questionnaire. Participants made daily observations of birds (study population) present at their feeders each month (considered one observation site). They could make observations for as many or as few days as they wished each month. Participants monitored the presence of both healthy and conjunctivitis-affected birds each day of observation. Comments, especially descriptions of clinical signs and behavior of diseased birds of all monitored species, were requested from participants. Reports of other diseased species also were solicited. No attempt was made to confirm suspected MG infections by diagnostic testing, tracking individuals, or counting diseased birds.

The daily observations of birds were collapsed into cumulative monthly values by Laboratory of Ornithology staff. Each species' monthly disease status at a particular site consisted of two categories: "healthy" and "conjunctivitis". A "healthy" status consisted of daily observations of normal appearing birds only. A "conjunctivitis" status consisted of at least one daily observation of an abnormal appearing bird, as well as any normal birds seen (if any).

The types of bird feeders in use for each site were provided monthly by participants, including ground, platform, hopper, tube, and small satellite types. Information on other site char-

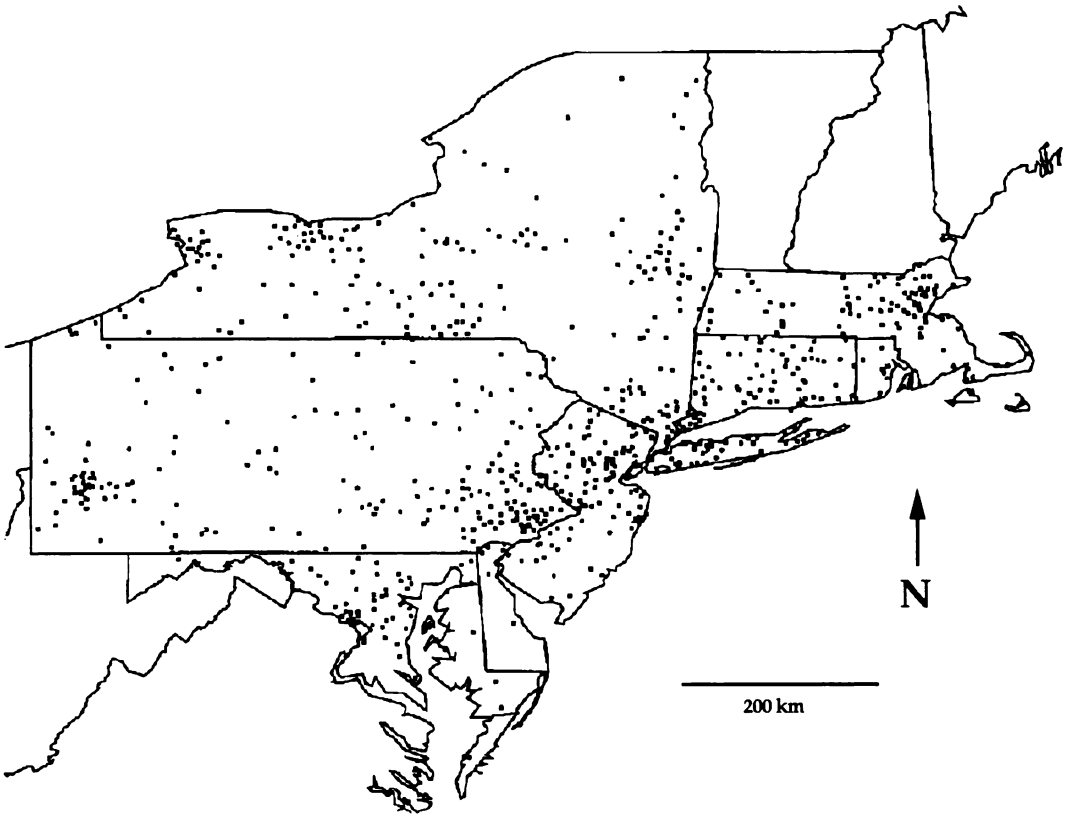

FIGURE 1. Distribution of observers involved in a study of conjunctivitis of house finches from an eight state region of the eastern USA.

acteristics were obtained from the volunteers' Project FeederWatch records and corresponded to the year of participation. This included the number of bird feeders present (≤ versus > median per site), the neighborhood housing locale (rural, rural/suburban mix, suburban, or urban), and altitude of the site.

The data were stratified by year of collection; November 1994 through October 1995 was considered year 1 (YR1), and November 1995 through October 1996 was considered year 2 (YR2). Months of the year were stratified according to the annual cycle of eastern house finches, based on the description by Hill (1993), including winter (November through February), spring migration (March), breeding (April through June), molting (July and August), and dispersal and fall migration (September and October) periods.

The overall occurrence of conjunctivitis in the five species were compared by using unadjusted odds ratios and Chi-square tests (Kelsey et al., 1996). The significance of association between the individual factors and the disease status of house finches were evaluated using Chi-square tests. The factors included, year, season, presence of the various feeder types, number of feeders, housing locale and altitude.

A stepwise logistic regression analysis was then used to evaluate the potential predictive association of the study variables with the presence of diseased house finches (Hosmer and Lemeshow, 1989). An additional variable (Start) was introduced to control for possible observer bias from YR1 to YR2 due to differential reporting of conjunctivitis by new participants beginning in November 1995. The first month of participation was dichotomized to those participants who began at any point in YR1 (referent) and those beginning during YR2. In addition, seasons were grouped into a high risk period (occurrence of conjunctivitis ≥30%, September to March) and a low risk period (April to August). Interactions among study variables were excluded from the logistic model due to potential difficulty in their biological interpretation. This analysis allowed for examination of an OR for each variable, adjusting for effects of all other variables remaining in the model, after stepwise removal of

non-significant factors (the computer-driven removal and entry limits were $P > 0.15$ and <0.10, respectively; BMDP statistical software, version 7.0, Los Angeles, California, USA). Significance was established at $P < 0.05$.

Because the observations of house finches were clustered by observer, and each observer made several observations in our study, we postulated that clustering may have been correlated with the likelihood of reporting conjunctivitis in the study population. This correlation between responses (conjunctivitis) occurs because they are dependent on exogenous factors that are associated with these responses. Conditioning on the observed set of these factors, and controlling for their effect in the analysis by including them as covariates in the logistic regression analysis, may achieve conditional independence. More often, however, this correlation in the responses arises from both observed and unobserved risk factors. In our analysis above, we assumed that the unobserved risk factors were randomly distributed among observers, and that there was no clustering of reporting of conjunctivitis by observer. We evaluated the overall significance of this assumption by using a mixed effect logistic regression model (Rosner, 1982). The mixed effect logistic regression model was specified as $P(CP/\alpha,\beta_i,\sigma) = 1/(1 + \exp - (\alpha + \Sigma\beta_i Z_i + \mu_i\sigma))$, where $P(CP/\alpha,\beta_i,\sigma)$ is the probability that conjunctivitis was reported by an observer μ_i given a set of fixed factors Z_i (year, season, platform, tube, start) with an effect of β_i. The likelihood ratio test was used to evaluate the significance of the observer random effect parameter in the mixed logistic regression analysis (EGRET Statistical Software, 1990 version; Statistic and Epidemiology Research Corporation, Seattle, Washington, USA).

RESULTS

Participants provided 7,224 monthly observations for the 24 mo period of the study. There was no unexpected clustering of observers in the study area (Fig. 1). The mean (±SE) number of observations per participant was 9.3 ± 0.25. Sixty percent (4,323 of 7,224) of observations were made in YR1; 40% (2,901 of 7,224) were made in YR2. The mean number of daily recordings contributing to each monthly observation was 16.9 ± 0.1. The proportion of disease reported by observers whose monthly reports were based on ≤1 wk (lowest quartile) versus ≥25 days (top quartile) were not significantly different by Chi-square.

TABLE 1. Comparison of conjunctivitis observed in eastern house finches versus other passerine species in the eastern USA.

Species	Conjunctivitis	Normal	OR[a]	95% CI[b]
House finch	1,887	4,722	—	—
Purple finch	39	1,409	14.3	10.3–20.2
Chickadee	35	6,340	72.4	51.1–102.9
House sparrow	104	4,294	16.5	13.4–20.3
Junco	27	4,676	69.2	46.6–103.5

[a] Odds ratios compare the odds of conjunctivitis in house finches versus species listed.
[b] Confidence interval.

Site information from Project FeederWatch records provided additional details for 98% (7,078 of 7,224) of the monthly observations. The mean number of feeders operated at each site was 4.6 ± 0.03, the median was four. Approximately equal numbers of observations were conducted in rural (30%; 2,101 of 7,065), rural/suburban mix (36%; 2,591 of 7,065) and suburban (31%; 2,171 of 7,065) habitat. Only 3% (202 of 7,065) of observations were made in urban areas. Nearly all observations occurred at altitudes <750 m (91%; 5,978 of 6,546).

Conjunctivitis in house finches was significantly more common than in the other species surveyed (Table 1). Collectively, house finches were 14 to 72 times more likely to be observed with conjunctivitis than the other species ($P < 0.001$) across all sites.

Chi-square analyses identified significant associations between five survey variables and disease status in house finches (Table 2). Year of the study ($P < 0.001$), season ($P < 0.001$), and the presence of platform ($P = 0.004$), hopper ($P = 0.05$) and tube type feeders ($P = 0.005$) were significantly associated with disease status. Ground and satellite feeders, the number of feeders per site, neighborhood housing locale and altitude of the site were not as-

TABLE 2. Analysis of associations between potential risk factors and disease status (conjunctivitis) in house finches from the eastern USA.

Factor	Conjunctivitis	Normal	Chi-square	df	*P*
Year			66.5	1	<0.01
YR 1	996	3,005			
YR 2	891	1,717			
Season			22.1	4	<0.01
Winter	1,031	2,443			
Spring	255	561			
Breeding	327	962			
Molt	55	223			
Fall	219	533			
Ground feeder			1.7	1	NS[a]
No	797	2,077			
Yes	1,090	2,645			
Platform feeder			8.4	1	<0.01
No	1,103	2,575			
Yes	784	2,147			
Hopper feeder			3.7	1	0.05
No	637	1,705			
Yes	925	2,202			
Tube feeder			7.9	1	<0.01
No	523	1,475			
Yes	1,364	3,247			
Satellite feeder			<0.01	1	NS
No	1,516	3,790			
Yes	371	932			
Number of feeders			0.9	1	NS
4 or less	1,016	2,614			
More than 4	826	2,017			
Housing density			4.3	3	NS
Rural	494	1,325			
Rural/suburban	712	1,710			
Suburban	589	1,443			
Urban	62	127			
Altitude			1.7	1	NS
≤750 m	1,596	3,877			
>750 m	139	386			

[a] Not significant.

sociated with disease status in house finches.

Further inspection of the data revealed that participants who began in YR2 reported a significantly greater proportion of conjunctivitis in house finches than observers that began in YR1 reported for the same time period (OR = 1.5, $P < 0.01$). This suggested a systematic bias in reporting by the new observers, possibly due to publicity or recruitment of participants in areas where diseased finches were most evident or both.

Odds ratios for variables from the stepwise logistic regression model adjusting for the bias in YR2 observations were generally consistent with the bivariate results (Table 3), and suggest the second year of

TABLE 3. Results from the stepwise logistic regression model for the effects of variables on conjunctivitis in house finches from the eastern USA.

Variable	Coefficient	SE	OR[a]	95% CI[b]
Year	0.417	0.057	1.5	1.4–1.7
Season	0.271	0.064	1.3	1.2–1.5
Platform	−0.237	0.058	0.8	0.7–0.9
Tube	0.216	0.064	1.2	1.1–1.4
Start	0.510	0.158	1.7	1.2–2.3
Constant	−1.083	0.064	—	—

[a] Adjusted odds ratio.
[b] Confidence interval.

the study (95% confidence OR = 1.4–1.7), cooler periods from September through March (OR = 1.2–1.5) and tube type feeders (OR = 1.1–1.4) were potential risk factors for conjunctivitis in house finches at residential feeding sites. In addition, the presence of platform feeders may have been protective, as diseased house finches were less likely to be observed at sites with these feeders (OR = 0.7–0.9).

The random effect parameter of the mixed effect logistic regression analysis was not significant, suggesting there was no significant clustering of reporting of conjunctivitis by observer nor extrabinomial variation in the data.

DISCUSSION

Observations of wild birds and controlled transmission studies early in this epizootic suggested mycoplasmal conjunctivitis was limited to house finches, and only rarely observed in sympatric passerines (Porter, 1994). The HFDS used longitudinal observations of fluctuating populations over a broad geographic area to successfully confirm that conjunctivitis in house finches was significantly more common than in four sympatric species, presumably due to MG infection (no cases were confirmed by standard diagnostic methods). This study does not represent a classic observational prevalence or incidence study in which repeated measures or survival analysis can be performed because individuals were neither enumerated nor followed. The HFDS does, however, represent a novel approach for grossly assessing the magnitude and spread of an easily recognized disease syndrome in a wild species. Such studies are helpful in guiding future disease research and prescriptive management efforts aimed at disease prevention in wild populations.

There are potential pitfalls, such as inconsistent data collection and reporting, when using volunteers for scientific data gathering. We are confident, however, of the strong motivation of HFDS volunteers based on past performance in Project FeederWatch and other citizen-science projects administered by the Cornell Laboratory of Ornithology. We also believe the written instructions provided to each participant helped to standardize the training that each observer received, thereby minimizing interobserver variation and the introduction of bias. In our study, we controlled for significant observation bias from participants that began in YR2 in the logistic regression model (Start; Table 3). Alternatively, participants that dropped from the study during YR1 may have under-reported disease and lowered estimates of disease in YR1 compared to YR2. However, the health status of house finches was not significantly different between YR1 observers that dropped out prior to YR2 versus those that continued with the study. An independent analysis of observations from HFDS data using records from long-standing participants (≥16 months) showed an increasing occurrence of disease over time, consistent with our findings.

Conjunctivitis in house finches continues to spread geographically from east to west in a propagating epizootic fashion in areas where the disease has recently become established. In the mid-Atlantic states represented in this study, house finches were more likely to be observed with conjunctivitis during the second year (the third year of the outbreak), suggesting an increase in the prevalence of the disease during that time.

Conjunctivitis was more likely to be

found in annual periods for house finches characterized by greater ranging and contact with birds of disparate populations, formation of feeding and roosting aggregations, colder temperatures, and reliance on feeding stations (Belthoff and Gauthreaux, 1991; Hill, 1993). Independently or combined, these factors would likely result in crowding, increased use of common food sources, and changes in nutritional and metabolic status that would tend to favor transmission of MG from infected to susceptible hosts (O'Connor, 1996; Fischer et al., 1997).

Although house finch population size peaks during the breeding and molting periods in this region, our results indicate that the potential risk of conjunctivitis is lowest at this time. We speculate this is due to the scarcity of diseased birds from heavy mortality in wintering populations (limiting sources for new infection) as well as restricted ranging behavior due to breeding and nesting activity and less reliance on bird feeders (limiting exposure to remaining sources of MG) (Hill, 1993). Propagation of the epizootic during this period may be occurring by horizontal transmission of MG between excess unpaired males (house finch sex bias 1.5M: 1.0F, Hill, 1993) or vertically from persistent carriers to eggs and young as in poultry (Yoder, 1991; Gerlach, 1994), or both. It is unknown whether observations of diseased birds in the breeding and molting seasons are comprised primarily of roaming unpaired males. Additionally, field investigations are needed to confirm vertical transmission of MG in house finches.

As an initial investigation into the possible role of bird feeders as sources for transmission of MG, the odds of conjunctivitis observation in house finches were calculated over five common feeder types. Feeders may be significant for transmission either as a source of infectious agent (fomite) or as a focal point for diseased birds unable to successfully secure natural food sources, leading to increased direct contact events. Feeders with limited numbers of perches and greater crowding potential (tube types) were more likely associated with conjunctivitis observations, though all feeder types were used by diseased birds. Competition at tube feeders may lead to more frequent dominant-subordinate or agonistic encounters resulting in bird to bird contact (e.g. facial pecking). The seed source in this feeder type is also protected from the environment, possibly offering favorable microenvironments for MG protected in respiratory secretions that exposed surfaces do not offer (Quinn et al., 1994; Jordan, 1996).

More spacious feeding strategies using raised platforms likely allow for less crowded conditions, jostling between conspecifics and fewer direct interactions. Also, as the foodstuff is less concentrated and more exposed to the environment, the buildup of fragile MG organisms seems less likely. Microbiological sampling of feeders across time and varieties, though challenging because of the fastidiousness of MG, would help verify or reject these hypotheses.

Housing locale records were used to analyze disease risk in differing habitat types. Interestingly, conjunctivitis was no more or less likely in marginal, rural areas, than in preferred habitat (urban, suburban, suburban/rural mix areas).

In summary, increased odds of presumed MG-associated conjunctivitis in eastern house finches were associated with cooler, non-breeding periods from September to March and the presence of tube type feeders at residential sites in the eastern USA. Strategies to decrease these potential risks and help prevent transmission may include modifying bird feeding activities appropriately, based on season and type of feeders in use.

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