

Further Western Spread of *Mycoplasma gallisepticum* Infection of House Finches

David H. Ley,¹ Deborah S. Sheaffer,² and Andre A. Dhondt³

¹North Carolina State University, College of Veterinary Medicine, Raleigh, North Carolina, USA; ²Audubon Society of Portland, Wildlife Care Center, Portland, Oregon, USA; ³Cornell University, Laboratory of Ornithology, Ithaca, New York, USA

ABSTRACT

Mycoplasma gallisepticum (MG) an important pathogen of poultry, especially commercially produced chickens and turkeys, emerged in 1994 as the cause of conjunctivitis in house finches (*Carpodacus mexicanus*) in their eastern range of North America (4, 6, 12, 13). The resulting epidemic of MG conjunctivitis severely decreased house finch abundance (8), and the continuing epidemic in the eastern range has been associated with repeating seasonal peaks of conjunctivitis and limitation of host populations (1, 8). MG conjunctivitis was first confirmed in the western native range of house finches in 2002 in a Missoula, Montana population (5).

We report further western expansion of MG conjunctivitis in the native range of house finches based on positive polymerase chain reaction (PCR) results with samples from birds captured in 2004 and 2005 near Portland, Oregon (14). Furthermore, to explore the possibility of genomic variability among house finch MG isolates we selected samples from our archive of reference strains and wild songbird isolates to analyze using amplified-fragment length polymorphism (AFLP) (2). AFLP results confirmed previous observations that during the initial stages of the MG epidemic in songbirds, isolates from different geographic locations and songbird species had genotypes that appeared to be highly similar, further supporting a single point-source of origin (2, 12). One 2001 isolate from New York was clearly different from the other songbird samples indicating that substantial molecular evolution or a separate introduction has occurred (2).

Expansion of MG conjunctivitis in house finches to Oregon and evidence that the strains involved are showing genotypic variability are reminders that commercial poultry producers should maintain bio-security measures that minimize contact between songbirds, especially house finches, and poultry.

MG IDENTIFIED BY PCR IN OREGON HOUSE FINCHES

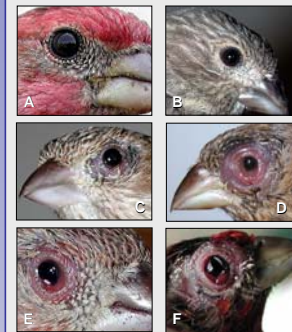
Wild-caught house finches submitted to the Audubon Society Wildlife Care Center (Portland, OR) having eye lesions compatible with MG conjunctivitis (Fig. 1) were evaluated (0-3 score based on severity of conjunctivitis), and sampled for mycoplasma culture and MG PCR (Table 1). Sterile swabs were used to sample conjunctival sacs of both eyes, and in one case the choanal cleft. Swabs were placed in 2 ml of Frey's mycoplasma broth media with 15% swine serum (9). Inoculated broths were stored at 4 C for up to 72 hours before overnight shipment on cold packs to NC State University College of Veterinary Medicine (Raleigh, NC). Upon arrival, 1 ml of inoculated broth from each sample was removed for PCR processing. The remaining inoculated broths were incubated at 37 C in humidified air for 4 to 5 weeks with 3 passages to additional Frey's broth and agar media to allow for mycoplasma growth (9).

No mycoplasmas were isolated in culture from any of the samples submitted. However, at least one sample from 3 of 4 house finches with eye lesions typical of MG conjunctivitis showed positive PCR results (Table 1). Conjunctival swabs from right and left eyes of 4 house finches and a choanal cleft swab from one of these birds were tested using the 16S rRNA gene PCR method described by Lauerma (11), resulting in 6 of 9 positive reactions. Additionally, right and left conjunctival swabs of the most recently sampled house finch (id. 144-M), which were both positive by the Lauerma method, were also positive using primers to the *mgc2* surface protein gene of MG. This method was recently described by Garcia (7) and reported to have increased specificity and sensitivity compared to the 16S rRNA PCR method.

The increasing reports of house finches in their native western range with eye lesions typical of MG conjunctivitis (3), observation of eye lesions in captured house finches by veterinary health care professionals, and positive PCR results using two different methods, are compelling evidence for the further western extension of the epidemic in house finches that was first observed and confirmed in 1994 in the eastern population.

Culture and isolation of MG organisms from west coast house finches has so far not been successful, most likely due to loss of viability during storage and transportation. We have found that culture and isolation success is improved when samples are incubated at 37 C immediately after broths are inoculated. Isolation of MG organisms from the western extension of this emerging disease is highly desirable because that would enable further exploration of the molecular epidemiology of the disease and the possible molecular evolution of the pathogen.

Fig. 1. Normal and Affected House Finches



Normal male (A) and female (B) house finches. House finches with periorbital swelling, inflammation, and conjunctivitis – typical clinical signs of MG disease in songbirds (C,D,E,F).

Table 1. MG PCR results from Oregon House Finches^a

| Accession no. | House finch no.-sex | Eye lesion score ^b | Samples collected ^c | MG PCR 16S rRNA ^d | <i>mgc2</i> ^e |
|---------------|---------------------|-------------------------------|--------------------------------|------------------------------|--------------------------|
| 2004.033 | 257-F | R = 0 L = 2 | 07 Apr 04 | - | nd |
| | 259-F | R = 3 L = 3 | 07 Apr 04 | + | nd |
| 2004.044 | 546-nr | R = 3 L = 3 | 19 May 04 | + | nd |
| | | | | + | nd |
| 2005.012 | 144-M | R = 3 L = 1 | 03 Aug 05 | + | + |
| | | | | + | + |

^aHouse finches with eye lesions typical of MG conjunctivitis were submitted to the Audubon Society of Portland, Wildlife Care Center, Oregon, USA.

^bEye lesions were scored for right (R) and left (L) eyes on a scale of 0-3 as follows: a zero score represented no signs of conjunctivitis; eyes with minor swelling or redness were assigned a 1; eyes with moderate swelling and discharge received a 2, and severely swollen eyes (those that were nearly or completely swollen shut) were classified as 3.

^cSamples collected were conjunctival swabs inoculated to modified Frey's mycoplasma broth with 15% swine serum.

^dPCR method to detect MG 16S rRNA gene (11).

^ePCR method to detect MG *mgc2* surface protein gene (7).

nr = no record.

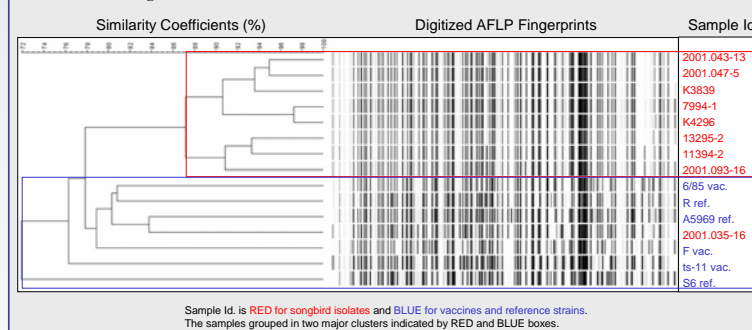
nd = not done.

Table 2. MG Isolates from Songbirds 1994-2001

| Isolate id. | Isolated (mo/yr) | Host species | Location | Isolated by |
|-------------|------------------|---------------|----------------|-------------|
| 7994-1 | 06/94 | House finch | Virginia | NCSU |
| 11394-2 | 07/94 | Blue jay | Virginia | NCSU |
| K3839 | 11/94 | House finch | Maryland | SCWDS/UGA |
| 13295-2 | 08/95 | House finch | North Carolina | NCSU |
| 1596-5 | 02/96 | Am. goldfinch | North Carolina | NCSU |
| K4269 | 07/96 | House finch | Ohio | SCWDS/UGA |
| 2001.035-16 | 04/01 | House finch | New York | NCSU |
| 2001.043-13 | 05/01 | House finch | Wisconsin | NCSU |
| 2001.047-5 | 05/01 | House finch | New York | NCSU |
| 2001.093-16 | 10/01 | House finch | Georgia | NCSU |

Selected MG isolates from three songbird species made in 1994 to 2001 from seven states of the USA. Two isolates (SCWDS/UGA) were provided by P Luttrell and JF Fischer (Southeastern Cooperative Wildlife Disease Study) and SH Kleven (Dept. Avian Med., UGA).

Fig. 2. AFLP analysis of MG Vaccines, Reference Strains, and Songbird Isolates



Sample Id. is RED for songbird isolates and BLUE for vaccines and reference strains. The samples grouped in two major clusters indicated by RED and BLUE boxes.

GENOTYPIC VARIABILITY OF HOUSE FINCH MG ISOLATES

Random amplification of polymorphic DNA (RAPD) demonstrated a single, unique RAPD profile among house finch and other songbird MG isolates, suggesting a single point source of origin and one 'strain' common to the outbreak (12). However, some genomic variability of MG house finch isolates was identified by PCR-RFLP and nucleotide sequencing of the *pvpA* gene (15). Recently, we have observed genomic variability among house finch MG isolates using RAPD (2). However, RAPD fingerprints are prone to variability and are difficult to reproduce and standardize, making interpretation challenging and subjective. To more precisely explore the possibility of genomic variability among house finch MG isolates we selected reference strains and songbird isolates to analyze using amplified-fragment length polymorphism (AFLP).

MG strains analyzed included vaccine strains F, 6/85 (Intervet Inc.), and ts-11 (Select Laboratories); and reference strains S6, R, and A5969. Also included were MG isolates from 10 wild-caught songbirds with conjunctivitis (Fig. 1). These included 6 birds captured between 1994-96 (1 blue jay, 1 American goldfinch, and 4 house finches), and 4 house finches captured in 2001 (Table 2). AFLP was conducted according to Kokotovic (10). Amplification fragments were detected on a 310 Genetic Analyzer (Applied Biosystems) and initial data collection and preprocessing were performed using Genescan analysis software (Applied Biosystems). Preprocessed densitometric curve data were imported to GelCompar 2.0 (Applied Maths BVBA) where similarity among samples was calculated using the band-based Dice similarity coefficient, and clustering of samples (dendrogram) was performed using the unweighted pair-group method with arithmetic averaging (UPGMA).

Fig. 2 shows AFLP results of MG vaccines (ts-11, 6/85, F), reference strains (S6, A5969, R), and 9 songbird isolates (Table 2, except 1596-5). AFLP analysis generated 50-80 bands per sample, which allows resolution of finer-scale quantitative variation among the samples.

All but one of the songbird isolates were grouped together and have similarity coefficients of 91.5 to 97%, and cluster at a linkage level of 87%, indicating that they are closely related. The vaccine and reference strains have similarity coefficients of 72 to 85.5%, confirming that they are different strains. One house finch isolate (2001.035-16) was clearly different from the other songbird isolates and grouped with the vaccine and reference strains, suggesting that substantial molecular evolution or a separate introduction of a 'new' MG strain occurred.

AFLP results support previous observations (12) that during the initial stages of the MG epidemic in songbirds, isolates had genotypes that appeared to be closely related. This indicated that the outbreak in various songbird species and geographic locations was caused by the same or closely related strain of MG, suggesting a single point-source of origin.

More extensive analyses of historical and contemporary isolates of MG from house finches and other songbirds, using improved genotyping techniques such as AFLP, may help answer questions about the epidemiology and molecular evolution of MG conjunctivitis.

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