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House Finch (*Haemorhous mexicanus*) Conjunctivitis, and *Mycoplasma* spp. Isolated from North American Wild Birds, 1994–2015

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Abstract

Sampling wild birds for mycoplasma culture has been key to the study of 'House Finch (*Haemorhous mexicanus*) conjunctivitis', yielding isolates of *Mycoplasma gallisepticum* spanning the temporal and geographic ranges of disease from emergence to endemicity. Faced with the challenges and costs of sample collection over time and from remote locations for submission to our laboratory for mycoplasma culture, protocols evolved to achieve a practical optimum. Herein we report making *M. gallisepticum* isolates from House Finches almost every year since the disease emerged in 1994, and we now have 227 isolates from 17 US states. Our wild bird host range for *M. gallisepticum* isolates includes Blue Jay (*Cyanocitta cristata*), American Goldfinch (*Spinus tristis*), Lesser Goldfinch (*Spinus psaltria*), Purple Finch (*Haemorhous purpureus*), Evening Grosbeak (*Coccothraustes vespertinus*), and herein first reports for Western Scrub-jay (*Aphelocoma californica*), and American Crow (*Corvus brachyrhynchos*). By collecting and identifying isolates from birds with clinical signs similar to 'house finch conjunctivitis' we also expanded the known host range of *Mycoplasma sturni* and obtained isolates from additional wild bird species. Accumulating evidence shows that a diverse range of wild bird species may carry or have been exposed to *M. gallisepticum* in the US, as in Europe and Asia. Therefore, the emergence of a pathogenic *M. gallisepticum* strain in House Finches may actually be the exception that has allowed us to identify the broader epidemiologic picture.

Keywords

Conjunctivitis; *Haemorhous mexicanus*; House Finch; *Mycoplasma gallisepticum*; *Mycoplasma gypis*; *Mycoplasma sturni*; mycoplasmosis; wild birds

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Mycoplasma gallisepticum is a well characterized bacterial pathogen of chickens and turkeys worldwide and was thought to be relatively host-specific and pathogenic primarily for gallinaceous birds (Raviv and Ley 2013), until 1994 when it was identified as the cause of epidemic conjunctivitis in Eastern US House Finches (*Haemorhous mexicanus*; Ley et al. 1996). Disease in House Finches expanded rapidly in the Eastern North American host range (Dhondt et al. 1998) with high prevalence (Altizer et al. 2004) and high mortality (>50% population declines; Hochachka and Dhondt 2000). Dispersal to the Western US host range took several years (Duckworth et al. 2003; Ley et al. 2006) with lower prevalence and mortality (Dhondt et al. 2006). While House Finches appeared to be the wild bird species primarily impacted by *M. gallisepticum* conjunctivitis there were reports of similar disease in other wild birds, most notably American Goldfinch (*Spinus tristis*), Purple Finch (*Haemorhous purpureus*; Hartup et al. 2000), Evening Grosbeak (*Coccothraustes vespertinus*) and Pine Grosbeak(*Pinicola enucleator*; Mikaelian et al. 2001). A succession of US National Science Foundation and National Institutes of Health grants provided us the opportunity to collect samples and study this emergent disease with a multi-institutional, multi-disciplinary team. This study has yielded a wealth of knowledge and has been a model of collaborative research(Dhondt et al. 2005). Key to the success and productivity of this effort has been the collection of *M. gallisepticum* isolates spanning the temporal and geographic ranges of the disease from emergence to endemicity in wild bird hosts. We here report compiled results of our wild bird sampling for mycoplasmas from the emergence of 'House Finch conjunctivitis' in 1994 to 2015. *Mycoplasma gallisepticum* isolates from House Finches have been made in almost every year since 1994, for a current total of 227 isolates from 17 US states (Table 1). We also found that House Finches can be infected with other *Mycoplasma*spp. and discovered a broader wild bird species range infected with *M. gallisepticum*, including this first report for American Crow and Western Scrub-jay. Also new and of interest are other mycoplasmas isolated from a range of wild bird species, primarily *M. sturni*, thus expanding the previously known host-species range for this organism.

*Mycoplasma*culture is optimized by doing everything possible to assure organism viability with minimal delays and proper storage from collection to incubation in mycoplasma growth medium (Kleven 2008). In our case, optimal conditions were difficult to meet by cooperators of varied experience from multiple locations without well equipped laboratories, and was further complicated by the resulting need for sample storage and shipment to the mycoplasma laboratory. Faced with the challenges and costs (mainly overnight shipment on dry ice or cold-packs) of sample collection for mycoplasma culture from multiple locations over long periods, our sampling protocol evolved with experience. Our current mycoplasma sampling and culture protocol specifies conjunctival swabs (sterile, nylon, or polyester tips, plastic handles) inoculated to mycoplasma transport media (BD/Copan UTM, BD, Sparks, Maryland, USA or Remel M4 or M5, Remel, Lenexa, Kansas, USA) stored at 4 C and overnight shipment on cold-packs to the mycoplasma laboratory where culture is initiated immediately upon arrival in Frey's medium with 15% swine serum incubated at 37 C (Kleven 2008). Even with this protocol, mycoplasma culture-positive rates are highly variable among samples submitted, but can attain 50–90% from House Finches with conjunctivitis.

Wild bird mycoplasma isolation and identification results since 1994 are summarized in Table 1. Most of these are *Mycoplasma* spp. culture isolates (now in archival storage at −70 C) with identification of *M. gallisepticum* and *M. sturni* by species-specific immunofluorescence of colonies (Ley et al. 1998; Kleven 2008). *Mycoplasma gallisepticum*-specific PCR (Garcia et al. 2005) and 16S rRNA gene sequencing (Ley et al. 2012) were also used in some cases for *Mycoplasma* spp. identification. The majority of our isolates are from House Finches, the original wild bird host of interest. We have *M. gallisepticum* isolates from House Finches almost every year since disease emergence in 1994 from Virginia, and now from 17 states for a total of 227 isolates. A 2015 House Finch sample from Colorado was *M. gallisepticum* PCR-positive, but culture was not successful. Surprisingly, we also isolated two other *Mycoplasma* spp. from House Finches. In 2006, we identified a House Finch isolate made in California as *M. sturni* that was not pathogenic by experimental infection (Ley et al. 2010) and remains a unique finding. In a 2015 California submission from three House Finches and one Red-tailed Hawk (*Buteo jamaicensis*), *M. gallisepticum* was isolated from a House Finch and *M. gypis* was isolated from the Red-tailed Hawk and the other two House Finches, a novel finding in this host. We also have unidentified isolates from California: three from House Finches, three from American Crows (*Corvus brachyrhynchos*), and one from a Red-shouldered Hawk (*Buteo lineatus*).

In addition to isolating multiple *Mycoplasma* spp. from House Finches, we have isolated *M. gallisepticum* and *M. sturni* from diverse wild bird species. Our *M. gallisepticum* isolate host range includes: House Finch, Blue Jay (*Cyanocitta cristata*), American Goldfinch, Lesser Goldfinch (*Spinus psaltria*), Purple Finch, Evening Grosbeak, Western Scrub-jay (*Aphelocoma californica*), American Crow, and Black-capped Chickadee (*Poecile atricapillus*; all molecular identification, not cultured). Our *M. sturni* isolate host range includes: House Finch, Blue Jay, Northern Mockingbird (*Mimus polyglottos*), European Starling (*Sturnus vulgaris*), American Crow, American Robin (*Turdus migratorius*), Carolina Wren (*Thryothorus ludovicianus*), Cliff Swallow (*Petrochelidon pyrrhonota*), and Barn Swallow (*Hirundo rustica*; all molecular identification, not cultured).

Selected *M. gallisepticum* isolates in experimental infections of House Finches showed evolution of virulence with parallel patterns of increased virulence in both Western and Eastern isolates (Hawley et al. 2013). Phylogenetic studies using our *M. gallisepticum* isolates from wild birds and poultry suggested at least two host transfers from poultry to house finches, but only one successful lineage accounting for the continent-spanning epidemic (Hochachka et al. 2013). *Mycoplasma gallisepticum* isolates from our collection showed extensive variation in surface lipoprotein gene content, phenotypic plasticity, and genomic changes (Tulman et al. 2012).

Accumulating evidence shows that the wild bird host range for *M. gallisepticum* is surprisingly diverse. Using serology and PCR, we recently found that a diverse range of wild bird species may carry or have been exposed to *M. gallisepticum* in the US (Dhondt et al. 2014), as in Europe (Pennycott et al. 2005) and Asia (Shimizu et al. 1979; Ganapathy et al. 2007). Infections of the broader host range of wild birds could represent further host switching by the House Finch clade or multiple lineages of *M. gallisepticum*. Additional work is needed to identify the phylogenetic relationships of *M. gallisepticum* strain(s)

infecting the entire array of wild bird host species. It is possible that *M. gallisepticum* detected in wild birds globally represents past or recent introductions from poultry, with 'non-commercials' (backyard) poultry being the most common reservoirs of diverse *M. gallisepticum* strains (McBride et al. 1991; Ewing et al. 1996; Thekisoe et al. 2003) to interface with wild birds.

Mycoplasmas are more often commensals than pathogens, causing subclinical and chronic or latent infections (Bradbury 2005; Citti and Blanchard, 2013). Evidence for a diverse wild bird host range infected with *M. gallisepticum* may be another example of transmissible subclinical mycoplasmosis, achievement of an ideal host/parasite relationship. Emergence of a pathogenic *M. gallisepticum* strain in House Finches may be the exception that has allowed us to identify the broader epidemiologic picture.

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Table 1

Mycoplasma spp. isolated and/or identified from wild bird samples from 17 US states (1994–2015). *Mycoplasma gallisepticum* and *M. sturni* isolates were identified by species-specific immunofluorescence. *Mycoplasma gallisepticum* identification was often confirmed by species-specific PCR.

Species	Year	Location[a]	*Mycoplasma* spp.	Number
House Finch (*Haemorhous mexicanus*)	1994–99, 2001–06, 2008–15	VA, DE, NC, GA, NY, MD, PA, TN, KY, OH, MI, MN, NJ, WI, CA, AL, OR, CO[a]	*M. gallisepticum*	227, 1[b]
	2006	CA	*M. sturni*	1
	2009, 2012	CA	Unidentified	3[c]
	2015	CA	*M. gypis*	2[d]
Blue Jay (*Cyanocitta cristata*)	1994	VA	*M. gallisepticum*	1
	1994	FL	*M. sturni*	1
Northern Mocking Bird (*Mimus polyglottos*)	1994	FL	*M. sturni*	1
American Goldfinch (*Spinus tristis*)	1996, 2006	NC	*M. gallisepticum*	5
European Starling (*Sturnus vulgaris*)	1997, 1998, 2002	MN, TN, GA	*M. sturni*	3
Purple Finch (*Haemorhous purpureus*)	1998, 2013	NY, VA	*M. gallisepticum*	3
Evening Grosbeak (*Coccothraustes vespertinus*)	1999	Quebec, Canada	*M. gallisepticum*	2
American Crow (*Corvus brachyrhynchos*)	1997, 2000, 2006, 2009, 2013–15	MN, WA, CA, OR	*M. sturni*	21
	2008, 2009	CA	Unidentified	3[c]
	2013, 2015[a]	CA	*M. gallisepticum*	1, 1[b]
American Robin (*Turdus migratorius*)	1997	MN	*M. sturni*	1
Carolina Wren (*Thryothorus ludovicianus*)	2003	NC	*M. sturni*	1
Red-shouldered Hawk (*Buteo lineatus*)	2006	CA	Unidentified	1[c]
Cliff Swallow (*Petrochelidon pyrrhonota*)	2011, 2012	CA	*M. sturni*	7
Black-capped Chickadee (*Poecile atricapillus*)	2012	NY	*M. gallisepticum*	1[e]
Lesser Goldfinch (*Spinus psaltria*)	2014, 2015	OR, CA	*M. gallisepticum*	2
Barn Swallow (*Hirundo rustica*)	2014	CA	*M. sturni*	4[f]
Red-tailed Hawk (*Buteo jamaicensis*)	2015	CA	*M. gypis*	1[d]
Western Scrub-jay (*Aphelocoma californica*)	2015	CA	*M. gallisepticum*	1

[a]VA = Virginia; DE = Delaware; NC = North Carolina; GA = Georgia; NY = New York; MD = Maryland; PA = Pennsylvania, TN = Tennessee; KY = Kentucky; OH = Ohio; MI = Michagen; MN = Minnesota; NJ = New Jersey; WI = Wisconsin; CA = California; AL = Alabama; OR = Oregon; CO = Colorado; FL = Florida; WA = Washington

[b] *Mycoplasma gallisepticum* identified by PCR but not isolated.

[c] *Mycoplasma* isolates that were not identified by the methods used herein.

[d] Mycoplasmas isolated in culture and identified by 16S rRNA gene sequencing.

[e] Mycoplasmas not isolated in culture but *M. gallisepticum* identified by PCR.

[f] Samples were not cultured but mycoplasmas identified by 16S rRNA gene sequencing.