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Mycoplasmosis of House Finches (*Haemorhous mexicanus*) and California Scrub-Jays (*Aphelocoma californica*) in a Wildlife Rehabilitation Facility with Probable Nosocomial Transmission

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ABSTRACT: We describe an investigation of an outbreak of conjunctivitis in juvenile House Finches (*Haemorhous mexicanus*) and California Scrub-jays (*Aphelocoma californica*) at a central California, US wildlife rehabilitation facility. In late May 2015, the facility began admitting juvenile finches, the majority with normal eyes at intake. In June, with juvenile finches already present, the facility admitted juvenile scrub-jays, all with normal eyes at intake. In July, after conjunctivitis was observed in increasing numbers of juvenile finches and scrub-jays, carcasses were submitted for postmortem examination. Histopathology of five finches and three scrub-jays identified lymphocytic infiltrates in the ocular tissues. Conjunctival swabs from 87% (13/15) finches and 33% (4/12) scrub-jays were PCR-positive for *Mycoplasma gallisepticum*. One finch and two scrub-jays were PCR-positive for *Mycoplasma synoviae*. Additionally, gene sequencing (16S ribosomal RNA and 16S-23S intergenic spacer region) identified *Mycoplasma sturni* from 33% (3/9) scrub-jays. This outbreak of conjunctivitis suggested that *M. gallisepticum*-infected juvenile finches admitted to and maintained in a multispecies nursery likely resulted in transmission within the facility to healthy juvenile finches and scrub-jays. Evidence of other *Mycoplasma* spp. in finches and scrub-jays indicates that these species are susceptible to infection and may act as carriers. This outbreak highlighted the need for effective triage and biosecurity measures within wildlife rehabilitation facilities.

Key words: California Scrub-jay, House Finch, *Mycoplasma gallisepticum*, *Mycoplasma sturni*, *Mycoplasma synoviae*, wildlife rehabilitation facility.

A wildlife rehabilitation facility in central California, US, admitted 8–10 adult House Finches (*Haemorhous mexicanus*) with conjunctivitis between February and April 2015. In late May and continuing through July 2015,

with none of the adult finches present, the facility admitted nestling to near-fledgling finches. The majority had normal eyes at intake; the few with conjunctivitis at intake were euthanized upon admission. Apparently healthy juvenile finches developed conjunctivitis 6–10 d (mean $\pm$ SE: 6.3 ± 1.2 ; $n=7$) after admission; early in the outbreak some were treated with gentamicin ophthalmic solution prior to euthanasia. Between June and July 2015, the facility admitted nestling to near-fledgling California Scrub-jays (*Aphelocoma californica*), all with normal eyes at intake, and housed them in a multispecies nursery that included the juvenile finches. Juvenile scrub-jays developed conjunctivitis 5–46 d (24.8 ± 3.6 ; $n=12$) after admission. In total, the center admitted 286 finches and 270 scrub-jays between January and December 2015. Ultimately, all birds exhibiting conjunctivitis were deemed nonreleasable and were euthanized.

In July 2015, after observing increased incidence of conjunctivitis in juvenile finches and scrub-jays in their nursery, the facility submitted frozen (-20°C) carcasses of 15 finches and 12 scrub-jays to the California Department of Fish and Wildlife's Wildlife Investigations Laboratory (WIL; Rancho Cordova, California). Of these, five finches (A–E) and three scrub-jays (A–C) were thawed overnight at 4°C and submitted to the California Animal Health and Food Safety Laboratory (Davis, California) for postmortem examination. Gross lesions in the five finches included conjunctivitis, dark-pink wet lungs, and red-tinged fluid in the trachea; sex was not determined. Histopathology on three of

these finches (A–C) identified multifocal lymphocytic infiltrates subtending the mucosal epithelial conjunctiva of the eyelids. Conjunctival and tracheal swabs were tested by real-time PCR (qPCR) for *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) using Idexx MG DNA and Idexx MS DNA kits (Idexx Laboratories, Westbrook, Maine, USA), respectively, according to the manufacturer's instructions; sequencing for the detection of other *Mycoplasma* spp. was not performed. Molecular diagnosis for MG and MS was based on quantification cycle (Cq) according to the manufacturer's instructions where reaction with $Cq \leq 40$ was positive, $Cq = 40$ was suspect, and no Cq was negative. Finches C, D, and E were positive and A and B were suspect for MG; all were negative for MS (Table 1). Aerobic bacteria by culture, *Chlamydophila* spp. by fluorescent antibody, and *Salmonella* spp., West Nile virus, and avian influenza by PCR were not detected.

Gross findings for the three scrub-jays (A–C) included wet periocular feathers, unilateral swollen eyelids, wet lungs, and enlarged livers, spleens, and bursas of Fabricius. Scrub-jay A was male; B and C were females. Microscopically, there were necrotic submucosal lymphoid tissue infiltrates in the eyelids. The spleen of scrub-jay A and the vascular lumina of scrub-jay C contained numerous leukocytes with intracellular protozoa (presumptive *Leucocytozoon* spp.). All three scrub-jays were negative for MG; scrub-jay A was positive and B and C were negative for MS (Table 1). *Enterococcus faecium* was detected by aerobic bacterial culture of a lung swab from scrub-jay B. *Chlamydophila* spp., *Salmonella* spp., West Nile virus, and avian influenza were not detected.

Of the remaining 10 finches (F–O) and nine scrub-jays (D–L) retained at WIL, the carcasses were thawed overnight at 4 C and eye lesions scored 0–3: 0=normal, 1=mild, 2=moderate, and 3=severe (Sydenstricker et al. 2006). Three swabs per bird (left and right conjunctiva, choana) were collected with flocked nylon swabs (FLOQSwabs, Copan Diagnostics, Murrieta, California, USA) and

shipped overnight on cold packs to the Mycoplasma Diagnostic and Research Laboratory, North Carolina State University (Raleigh, North Carolina) for molecular diagnostics. At the Mycoplasma Diagnostic and Research Laboratory, swabs were stored overnight at 4 C. Swabs from each bird were pooled and inoculated into 0.8 mL of phosphate-buffered saline. The DNA was extracted from 0.2 mL of each pooled phosphate-buffered saline sample and purified (QIAamp DNA Mini Kit, Qiagen, Valencia, California, USA). Real-time PCR for MG and MS was performed using Idexx MG DNA and Idexx MS DNA kits (Idexx Laboratories), respectively, according to the manufacturer's instructions. Real-time PCR for *Mycoplasma* spp. was performed using primers to the 16S ribosomal RNA gene and 16S-23S intergenic spacer region (Ley et al. 2012). Amplicons were sequenced (Eton Bioscience, San Diego, California, USA) and compared with 16S ribosomal RNA and nucleotide collection sequences, respectively, in GenBank.

Finches typically had bilateral conjunctivitis with a mean eye lesion score (left eye+right eye) of 3 ± 0.3 ($n=10$) while scrub-jays had unilateral conjunctivitis with a mean eye lesion score of 2 ± 0.3 ($n=9$; Table 1). *Mycoplasma gallisepticum* was identified from all 10 finches and four of nine scrub-jays; four scrub-jays were suspect for MG (Table 1). Also identified were MS and *Mycoplasma sturni* (Table 1).

Conjunctivitis caused by MG first emerged in 1994 in free-ranging House Finches in their introduced range in eastern US (Ley et al. 1996) and, by 2004, MG had been isolated from House Finches in their native western range (Ley et al. 2006). Since emergence, MG outbreaks have been recognized annually in House Finch populations (Hartup et al. 2001), and MG has been isolated from a diversity of wild bird species (Ley et al. 2016). In the MG outbreak investigated here, admissions of juvenile finches did not overlap temporally with adults in the rehabilitation facility; as such, it is probable that at least some juveniles were infected by their parents in the nest prior to admission (Hartup and Kollias 1999).

TABLE 1. Eye lesion scores and *Mycoplasma* spp. identified from House Finches (*Haemorrhous mexicanus*) and California Scrub-jays (*Aphelocoma californica*) during a conjunctivitis outbreak in a wildlife rehabilitation facility in central California, USA, between May and July 2015. *Mycoplasma gallisepticum* and *Mycoplasma synoviae* were identified by species-specific real-time PCR (qPCR), where quantification cycle (Cq): <40=positive, 40=suspect, no Cq=negative. Sequencing for the detection of other *Mycoplasma* spp. was performed at the MDRL using primers to the 16S ribosomal (r)RNA and 16S-23S intergenic spacer region. Molecular diagnosis was based on the combination of qPCR and sequence results.

Species	ID	Eye lesion score ^a	Mycoplasma diagnosis ^b					
		Left, right	Diagnostic lab ^c	MG qPCR	MS qPCR	16S rRNA sequence	ISR sequence	Molecular diagnosis
House Finch	A	ND	CAHFS	Suspect	Negative	ND	ND	MG suspect
	B	ND	CAHFS	Suspect	Negative	ND	ND	MG suspect
	C	ND	CAHFS	Positive	Negative	ND	ND	MG
	D	ND	CAHFS	Positive	Negative	ND	ND	MG
	E	ND	CAHFS	Positive	Negative	ND	ND	MG
	F	3, 1	MDRL	Positive	Negative	MG	MG	MG
	G	1, 1	MDRL	Positive	Negative	ND	MG	MG
	H	1, 2	MDRL	Positive	Negative	MG	MG	MG
	I	1, 2	MDRL	Positive	Suspect	MG	MG	MG/MS suspect
	J	2, 1	MDRL	Positive	Suspect	MG	MG	MG/MS suspect
	K	0, 2	MDRL	Positive	Positive	MG	MG	MG/MS
	L	3, 2	MDRL	Positive	Negative	MG	MG	MG
	M	2, 2	MDRL	Positive	Negative	MG	MG	MG
	N	1, 1	MDRL	Positive	Negative	MG	MG	MG
	O	1, 1	MDRL	Positive	Negative	ND	MG	MG
California Scrub-jay	A	ND	CAHFS	Negative	Positive	ND	ND	MS
	B	ND	CAHFS	Negative	Negative	ND	ND	Not determined
	C	ND	CAHFS	Negative	Negative	ND	ND	Not determined
	D	1, 1	MDRL	Suspect	Negative	ND	PQ	MG suspect
	E	1, 1	MDRL	Positive	Negative	Mst	MG	MG/Mst
	F	1, 1	MDRL	Suspect	Negative	ND	PQ	MG suspect
	G	3, 0	MDRL	Positive	Negative	ND	MG	MG
	H	0, 2	MDRL	Suspect	Negative	Mst	MG	MG/Mst
	I	1, 0	MDRL	Suspect	Negative	ND	PQ	MG suspect
	J	1, 0	MDRL	Negative	Negative	Mst	pq	Mst
	K	1, 0	MDRL	Positive	Negative	ND	MG	MG
	L	1, 3	MDRL	Suspect	Positive	MS	PQ	MG suspect/MS

^a Eye lesion score: 0 = normal; 1 = mild; 2 = moderate; 3 = severe; ND = not done.
^b ISR = intergenic spacer region; ND = not done; MG = *M. gallisepticum*; MS = *M. synoviae*; PQ = poor quality; Mst = *Mycoplasma sturni*.
^c CAHFS = California Animal Health and Food Safety Laboratory (Davis, California); MDRL = Mycoplasma Diagnostic and Research Laboratory (Raleigh, North Carolina).

The lack of clinical signs at intake prompted rehabilitation personnel to place the juvenile finches in the nursery where juvenile House Sparrows (*Passer domesticus*), scrub-jays, American Crows (*Corvus brachyrhynchos*), Yellow-billed Magpies (*Pica nuttalli*), Northern Mockingbirds (*Mimus polyglottos*), American Robins (*Turdus migratorius*), and European Starlings (*Sturnus vulgaris*) were housed. In the nursery, up to six similar-aged juveniles of a single species were contained in a plastic mesh enclosure (56×41×25 cm) on shelving along two near-perpendicular walls. Enclosures were stacked four-high and two-wide for each of the species, which helped expedite the multiple feedings required each

day. Each enclosure was disinfected with a quaternary ammonium compound between groups of juveniles and separate food and feeding utensils were assigned to each group. However, the juveniles within each species were fed consecutively, always beginning with House Finches. Transmission of MG has been demonstrated to occur through direct contact (Farmer et al. 2002), aerosol (Beard and Anderson 1967), and fomites (Dhondt et al. 2007a). The multispecies nursery would have allowed many opportunities for the transmission of MG from infected juvenile finches to uninfected juvenile finches (Dhondt et al. 2007b) and scrub-jays, and possibly to other species. Although the rehabilitation facility has biosecurity protocols to minimize transmission of pathogens between groups of juveniles and among the different species, the placement of multiple species into a single room likely increased the probability of cross-species transmission and disease outbreak. Ideally, species at high risk for MG, such as House Finches, should be housed separately from other avian species. When possible, new admissions should be isolated for 30 d (Dhondt et al. 2008) before placement with the general population. In situations where birds cannot be effectively isolated, to prevent nosocomial infections euthanasia should be considered for species at high risk for MG infection.

Conjunctivitis was not observed in the other avian species housed in the nursery, although no sampling was conducted. Thus, the source of the *M. sturni* and MS remains undetermined. *Mycoplasma sturni* has been isolated previously from crows, robins, and starlings with and without conjunctivitis (Forsythe et al. 1996; Wellehan et al. 2001). During the outbreak in the rehabilitation facility, *M. sturni* was identified from three scrub-jays. The fact that the scrub-jays had clinical signs suggests they are susceptible to infection; however, two were coinfecting with MG. Research is needed to determine if clinical *M. sturni* infections occur in free-ranging scrub-jays.

The identification of MS is surprising because MS is primarily known to infect domestic poultry and has only been isolated from Wild Turkeys (*Meleagris gallopavo*; Fritz

et al. 1992), although the findings presented here suggested some passerine species may be susceptible. *Mycoplasma synoviae* was identified in one finch and two scrub-jays with conjunctivitis; however, one finch and one scrub-jay were coinfecting with MG. It is unknown if MS infection alone would result in conjunctivitis or if finches and scrub-jays may act as subclinical carriers. Although MS was identified in a single scrub-jay with conjunctivitis, the presence of mycoplasmas other than MG was not evaluated in this individual. The use of species-specific PCR for MG and MS, combined with *Mycoplasma* genus PCR and gene sequencing, is important for the detection of multiple *Mycoplasma* spp. Experimental infection of disease-free birds may then help identify their roles as pathogens.

The outbreak of conjunctivitis in this rehabilitation facility highlights the need for best management practices such as those outlined in the Minimum Standards for Wildlife Rehabilitation (Miller et al. 2012). These practices should include effective triage and isolation procedures, cleaning and disinfection protocols, knowledge of species at risk for infections, and prompt investigation and diagnosis when a disease outbreak occurs. Instituting these practices may be challenging for facilities operating with minimal personnel on limited budgets; however, they are vital to maximize patient care. Since refining their management practices, including the complete isolation of House Finches from other avian species, the rehabilitation facility has experienced no further conjunctivitis outbreaks even though WIL continues to document annual mycoplasmosis outbreaks in free-ranging wild birds in California.

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