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Mycoplasma sturni from Blue Jays and Northern Mockingbirds with Conjunctivitis in Florida

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ABSTRACT: Northern mockingbirds (*Mimus polyglottos*) and blue jays (*Cyanocitta cristata*) in a Florida (USA) wildlife care facility developed clinical signs and gross lesions suggestive of the ongoing outbreak of *Mycoplasma gallisepticum* (MG) conjunctivitis in house finches (*Carpodacus mexicanus*) and American goldfinches (*Carduelis tristis*). Mycoplasmal organisms were cultured from conjunctival/corneal swabs of birds with sinusitis, conjunctivitis, and/or epiphora. All of the isolates tested were identified as *Mycoplasma sturni* by indirect immunofluorescence. *Mycoplasma sturni* as well as MG should be considered in the differential diagnosis of songbirds with conjunctivitis.

Key words: Blue jay, case reports, conjunctivitis, *Cyanocitta cristata*, *Mimus polyglottos*, *Mycoplasma sturni*, northern mockingbird.

Common causes of conjunctivitis in avian species include numerous viruses, some bacteria, and *Chlamydia psittaci* (Swayne, 1996; Williams, 1994). *Mycoplasma* spp. have been suspected of being an important cause of conjunctivitis in companion birds, but often without definitive diagnosis (Williams, 1994). *Mycoplasma gallisepticum* (MG) has been associated with conjunctivitis in farmed gamebirds (Cookson and Shivaprasad, 1994) and commercial layer chickens (Nunoya et al., 1995), but it has not been considered a natural pathogen of wild birds (Ley and Yoder, 1997).

Reports of free-ranging house finches (*Carpodacus mexicanus*) with conjunctivitis began in February 1994 (Fischer et al., 1997). *Mycoplasma gallisepticum* was identified as the putative etiologic agent (Ley et al., 1996; Luttrell et al., 1996) of naturally acquired infections, and also was isolated from a blue jay (*Cyanocitta cristata*) that developed conjunctivitis after being housed in a cage previously occupied by affected house finches (Ley et al., 1996). Since the first reports from the mid-Atlantic region of the USA, house finches with conjunctivitis have been observed in what now encompasses essentially the entire range of their eastern population (Fischer et al., 1997). In 1995, American goldfinches (*Carduelis tristis*) with conjunctivitis were observed in North Carolina (USA), and two isolates of MG were made from affected birds (Ley et al., 1997). This outbreak has increased awareness of MG as a cause of conjunctivitis.

To investigate the outbreak of epidemic conjunctivitis in songbirds, samples for mycoplasma culture and identification were solicited from cooperators with access to affected birds. Beginning in July 1994, northern mockingbirds (*Mimus polyglottos*) and blue jays with clinical signs and gross lesions suggestive of MG-conjunctivitis were noted at Wildlife Care Center (Fort Lauderdale, Florida, USA; 26°08′N, 80°08′W; D. L. Anderson, pers. commun.). All of the affected birds (Table 1) were admitted as abandoned nestlings or fledglings in normal condition with no clinical signs or gross lesions. However, clinical signs became apparent in some cases as soon as 2 to 3 days after admittance. In September 1994, conjunctival/corneal swabs were taken (in most cases, after initiation of antimicrobial therapy) from a group of birds with sinusitis, conjunctivitis, and/or epiphora (D. L. Anderson, pers. commun.). Swabs were inoculated directly to Frey's broth medium (Kleven and Yoder, 1989) supplemented with 15% inactivated swine serum (FMS). Inoculated broths were shipped to the

TABLE 1. *Mycoplasma sturni* isolates from songbirds developing conjunctivitis following admittance to a Florida wildlife care facility in 1994[a].

Host species	Date admitted (mo/day)	Mycoplasma isolated (mo/day)	*Mycoplasma* sp.[b]
Mockingbird	8/11	9/16	*M. sturni*
Mockingbird	7/11	9/15	*M. sturni*
Mockingbird	8/1	9/16	NA[c]
Mockingbird	8/16	9/19	NA
Mockingbird	6/29	9/15	*M. sturni*
Mockingbird	5/31	9/15	*M. sturni*
Mockingbird	7/11	9/15	*M. sturni*
Blue jay	8/19	9/15	*M. sturni*
Blue jay	8/5	9/19	NA
Mockingbird	7/9	9/19	NA
Mockingbird	7/16	9/15	*M. sturni*

[a] FMS broths inoculated by conjunctival/corneal swabs for mycoplasma culture were received 13 September 1994.
[b] *M. sturni* identifications were made by indirect immunofluorescence using archived samples 6 November 1996.
[c] Not available.

North Carolina State University College of Veterinary Medicine (Raleigh, North Carolina, USA) with minimal storage and transit time at 4 C.

Inoculated broths (nine from northern mockingbirds and two from blue jays) were incubated at 37 C in a humidified incubator. Passages from FMS broth to agar were made to allow for colonial growth. Organisms isolated from conjunctival/corneal swabs had growth characteristics in broth and on FMS agar that were consistent with mycoplasmas (Class Mollicutes). The organisms were strong glucose fermentors in FMS broth and fast growing, resulting in reduced pH and color change of this medium between 24 to 48 hr of incubating the original field samples. Prevalence of the organism in the samples submitted (Table 1) was 100% (n = 11). Subsequent passages in FMS broth resulted in more rapid and abundant growth.

Species identification of mycoplasma colonies on FMS agar was attempted by direct immunofluorescence (Kleven and Yoder, 1989) using fluorescein-conjugated rabbit antisera provided by S. H. Kleven (Department of Avian Medicine, University of Georgia, Athens, Georgia, USA). Mycoplasmal colonies isolated from each of eleven samples submitted did not fluoresce when tested with conjugates for *M. gallisepticum, M. synoviae* and *M. meleagridis,* the most common pathogenic avian *Mycoplasma* spp. One isolate was tested with all additional conjugates (*M. gallinarum, M. gallinaceum, M. pullorum, M. iners, M. gallopavonis, M. iowae, M. cloacale, M. anatis,* and *Acholeplasma laidlawii*) available to us and was unreactive with each. Sterol requirement was assessed using the digitonin sensitivity test (Tully, 1983). Sensitivity to digitonin indicated a sterol requirement as would be expected of *Mycoplasma* spp., and not *Acholeplasma* spp. Therefore, these isolates from Florida northern mockingbirds and blue jays were originally recorded as unidentified mycoplasmas. In September, 1994 a subset of the isolates (6 mockingbird, one blue jay) was placed in archival storage at −80 C.

In 1996–97 a new *Mycoplasma* sp., *M. sturni,* isolated in Connecticut (USA) from a European starling (*Sturnus vulgaris*) with bilateral conjunctivitis, was described (Forsyth et al., 1996; Frasca et al., 1997). This finding suggested the possibility that the previously unidentified mycoplamas from northern mockingbirds and blue jays in Florida might be *M. sturni.* In November 1996, these isolates were removed from archival storage, cultured on FMS agar, and tested by indirect immunofluorescence (Kleven and Yoder, 1989) with antiserum prepared to *M. sturni* type strain UCMF (American Type Culture Collection No. 51945) (Forsyth et al., 1996). Briefly, mycoplasma colonies on FMS agar were incubated with *M. sturni* antiserum for 30 min at 37 C, then washed three times with PBS (pH 7.2). Monoclonal anti-rabbit immunoglobulins-FITC conjugate (Sigma Chemical, St. Louis, Missouri, USA) was incubated for 30 min at 37 C, and colonies were washed three times. The treated colonies were examined

microscopically (Labophot, Nikon Inc., Melville, New York, USA) under low magnification using ultraviolet light and epi-illumination. Each of the seven isolates tested showed fluorescence. Therefore, we now consider these isolates to be positively identified as unmixed cultures of *M. sturni*.

Initial attempts to identify the mycoplasmas isolated were not successful because none of the *Mycoplasma* sp.-specific antibody conjugates available to us at the time reacted with colonies of the organisms. It was apparent that the mycoplasmal organisms isolated were not MG based on results of the direct immunofluorescence tests. Growth characteristics of the mycoplasmas also suggested that these organisms were not likely to be the MG strain causing epidemic conjunctivitis. Under natural conditions MG has been isolated only from American goldfinches and house finches, and it is notably difficult to isolate and slow growing. In contrast, these isolates from northern mockingbirds and blue jays were easily isolated; they were fast growing and cultured with high prevalence (100%) from affected birds. However, definitive diagnosis of these isolates as *M. sturni* awaited testing with antiserum made to this newly characterized *Mycoplasma* sp.

It should be noted that the possible involvement of MG or other *Mycoplasma* spp. in these cases cannot be conclusively ruled-out based solely on failure to isolate organisms. As previously discussed (Cobb et al., 1992), there are numerous reasons why MG or other *Mycoplasma* spp. may not be isolated. For example, although immunofluorescence can identify *Mycoplasma* spp. in mixed culture, it may fail to do so if one species is present in very low numbers or if a fastidious species is masked by overgrowth of a more prolific species. Application of additional diagnostic methods such as serology and culture-independent organism detection assays such as *Mycoplasma* sp.-specific polymerase chain reactions could help define the role of *M. sturni* in future cases.

This is the first report of *M. sturni* isolation from a blue jay and the second report of isolation from northern mockingbirds (Frasca et al., 1997); it is additional evidence that *M. sturni* as well as MG should be considered in the differential diagnoses of songbirds with conjunctivitis. There are numerous questions regarding the significance of *M. sturni* isolations from songbirds such as host range, infectivity, virulence, transmissibility, etc., that need to be addressed to assess the possible pathogenic potential of this organism. For example, it will be important to demonstrate that *M. sturni* is capable of causing the disease that has been associated with its isolation, and whether there are strain variations among the various isolates. Assessment of the pathogenic significance of *M. sturni* will require experimental approaches involving potential host species, and surveillance involving field observations and isolation and identification of the organism.

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