Mycoplasmosis in Evening and Pine Grosbeaks with Conjunctivitis in Quebec

Authors: Mikaelian, Igor, Ley, David H., Claveau, Raynald, Lemieux, Michel, and Bérubé, Jean-Paul

Source: Journal of Wildlife Diseases, 37(4) : 826-830

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-37.4.826

Journal of Wildlife Diseases, 37(4), 2001, pp. 826–830

Mycoplasmosis in Evening and Pine Grosbeaks with Conjunctivitis in Quebec

Igor Mikaelian,[1,6,7] **David H. Ley,**[2] **Raynald Claveau,**[3] **Michel Lemieux,**[4] **and Jean-Paul Bérubé**[5] [1]Canadian Cooperative Wildlife Health Centre and Centre Québécois sur la Santé des Animaux Sauvages, 3200 Sicotte, C.P. 5000, Saint-Hyacinthe, Quebec, Canada, J2S 7C6; [2]College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough St., Raleigh, North Carolina 27606, USA; [3]Clinique vétérinaire Claveau-Dubord-Blue, 1310 Bd du Royaume, Chicoutimi, Quebec, Canada, G7H 5B1; [4]Ministère de l'environnement et de la faune, 605 de la Station, Shawinigan, Quebec, Canada, G9N 1V9; [5]2226 Lamarche, Jonquière, Quebec, Canada, G7S 1L4; [6]Present address: IDEXX Veterinary Services, 2825 Kovr Drive, West Sacramento, California 95605, USA; [7]Corresponding author (e-mail: igor-mikaelian@idexx.com).

ABSTRACT: An outbreak of conjunctivitis affected evening grosbeaks (*Coccothraustes vespertinus*) and pine grosbeaks (*Pinicola enucleator*) in Quebec (Canada) during the winter 1998–99. One to 30% of the individuals from these two species were sick at 13 feeding stations. Sick birds were thin and had unilateral or bilateral catarrhal and lymphoplasmacytic conjunctivitis and rhinitis, and mucopurulent infra-orbital sinusitis. Mycoplasmal organisms were isolated in cultures in an affected evening grosbeak and identified as *Mycoplasma gallisepticum* by direct immunofluorescence. Random amplified polymorphic DNA (RAPD) fingerprinting of this isolate resulted in a banding pattern that was identical to patterns of *M. gallisepticum* isolates made from similar lesions in house finches (*Carpodacus mexicanus*) and American gold finches (*Carduelis tristis*) throughout eastern North America. *Mycoplasma gallisepticum* was identified by polymerase chain reaction in another evening grosbeak and a pine grosbeak. These observations suggest that the same strain of *M. gallisepticum* is the likely etiology for the observed disease in evening and pine grosbeaks in Canada and represent an extension of the host-species range for the ongoing epidemic of *M. gallisepticum* conjunctivitis in eastern North America.

Key words: Case report, *Coccothraustes vespertinus*, conjunctivitis, evening grosbeak, mycoplasmosis, *Mycoplasma gallisepticum*, pine grosbeak, *Pinicola enucleator*.

Mycoplasma gallisepticum conjunctivitis recently has emerged as a disease of free-ranging house finches (*Carpodacus mexicanus*) in the eastern USA. The disease was first observed in suburban Washington D.C. and adjacent states (Ley et al., 1996) in 1994, and has since spread to house finches throughout their entire eastern range (Fischer et al., 1997; Dhondt et al., 1998), including southeastern Canada. The disease is now endemic in the eastern range of this species and is clinically characterized by conjunctivitis, sinusitis, and debilitation. A similar syndrome was described in purple finches (*Carpodacus purpureus*) from Virginia (USA; Porter 1994), and confirmed by Hartup et al. (2000) in New York (USA). *Mycoplasma gallisepticum* was subsequently isolated from free-ranging American goldfinches (*Carduelis tristis*) at numerous sites and from a captive blue jay (*Cyanocitta cristata*) (Ley et al., 1996; Ley et al., 1997). We herein report an outbreak of conjunctivitis and sinusitis in evening grosbeaks (*Coccothraustes vespertinus*) and pine grosbeaks (*Pinicola enucleator*) in Canada and the first isolation of *M. gallisepticum* from these species.

Pine grosbeaks (6 of 12) and evening grosbeaks (2 of 10) with conjunctivitis were first reported by JPB on 6 February 1999 at Saint-David-de-Falardeau (Quebec, Canada; 48°37′N, 71°07′W). Clinical signs ranged from mild to severe unilateral or bilateral ocular swelling. One pine grosbeak had severe dyspnea. A few pine grosbeaks were found dead in the vicinity of the feeding stations (Fig. 1) but were not submitted to the laboratory. Food was removed from the feeding station in an attempt to prevent the spread of the disease. However, birds were similar clinical signs were observed continuously until 27 February 1999, at which date food was made available at the feeding station in an attempt to catch diseased birds and submit them to the laboratory. This feeding sta-

FIGURE 1. A pine grosbeak (*Coccothraustes vespertinus*) with severe conjunctivitis and infra-orbital sinusitis. The bird was found dead in the the snow, a few meters from a feeding station.

tion was monitored 20 times from 6 February 1999 to 28 March 1999, for the presence of birds with conjunctivitis: 64 of 246 pine grosbeaks, and 15 of 115 evening grosbeaks had conjunctivitis. The prevalence did not seem to vary during this period (data not shown).

Subsequent cases were observed until May 1999 at this feeding station, and a feeding station at Saint-Félix-d'Otis (Quebec; 48°16′N, 70°37′W), but detailed information about these observations were not available. Similar cases also were recorded from 15 March 1999, to 15 April 1999 at 10 feeding stations within a radius of 30 km of Shawinigan (Quebec; 46°32′N, 72°46′W). The distance between Saint-Denis-de-Falardeau and Saint-Alexis-des-Monts (Quebec; 46°28′N, 73°08′W), the most distant sites where pine grosbeaks and evening grosbeaks with conjunctivitis were recorded, is 283 km. Ornithologists reported that 1 to 30% of evening grosbeaks and pine grosbeaks were affected at each feeding station in the Shawinigan area. Detailed information about the number of birds affected/present at this sites was not available. Other bird species, including house finches and purple finches, apparently remained unaffected. An undetermined number of evening grosbeaks and pine grosbeaks had conjunctivitis in February and March 2000 at two feeding stations at Ferland (Quebec; 48°06′N, 70°50′W) and at Saint-Félix-d'Otis.

Five evening grosbeaks and three pine grosbeaks with clinical signs and/or gross lesions were submitted to the Quebec Regional Office of the Canadian Cooperative Wildlife Health Centre (Saint-Hyacinthe, Quebec, Canada) in 1999. One evening grosbeak was submitted from each of the following sites in Quebec at Grand-Mère (Shawinigan area, 46°37′N, 72°42′W), Pointe-du-Lac (Shawinigan area, 46°17′N, 72°42′W), and Saint-Roch-de-Mékinac (Shawinigan area, 46°49′N, 72°46′W). Two evening grosbeaks and all three pine grosbeaks submitted to the laboratory were from Saint-David-de-Falardeau. Five birds were submitted dead. Three birds were submitted live and were euthanized with an intravenous administration of an euthanasia solution (T61, Hoechst Roussel Vet, Regina, Saskatchewan, Canada). Macroscopic lesions consisted in mild to severe unilateral (one of eight) to bilateral (seven of eight) conjunctivitis and infra-orbital sinusitis with serous and crusting ocular and nasal discharge. Fat reserves were totally depleted in all birds. Samples from major organs were taken for histology. The whole skull was sectioned in 3 mm thick sections for histologic examination in three birds. Specimens were fixed in 10% buffered formalin and were routinely processed for histopathology.

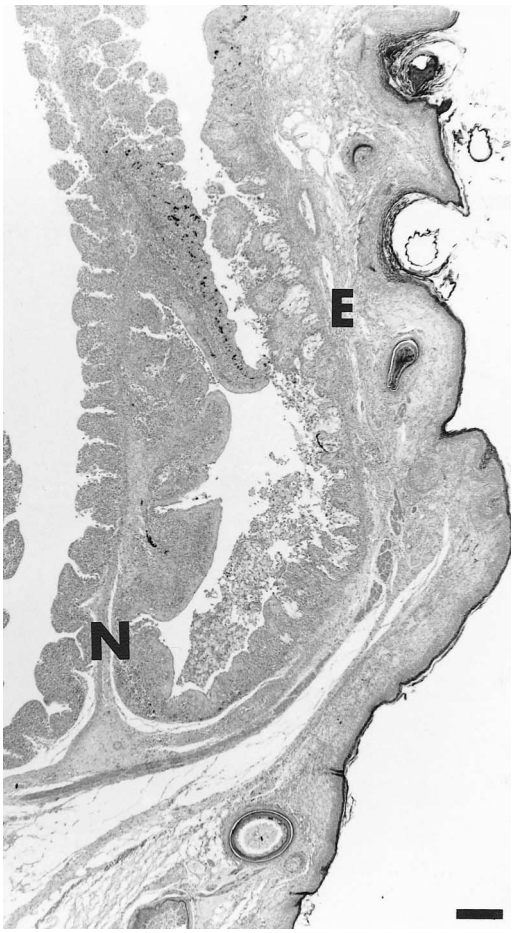

FIGURE 2. Lymphoplasmacytic conjunctivitis in a pine grosbeak (*Coccothraustes vespertinus*). The nictitating membrane (N) and the inner side of the eyelid (E) are markedly thickened by a dense inflammatory infiltrate. There is also marked hyperplasia of the conjunctival epithelium which is markedly contoured. Hematoxylin-phloxin-saffron. Bar = 150 μm.

The most prominent and consistent histologic change was moderate lymphoplasmacytic and heterophilic conjunctivitis with hyperplasia of the mucosal epithelium (Fig. 2). Corneal changes were present in two birds and consisted of moderate corneal edema, neovascularization, diffuse heterophilic infiltration and epithelial hyperplasia. Other changes included mucopurulent infra-orbital sinusitis (four of four), lymphoplasmacytic rhinitis (three of three), syalodacryoadenitis (two of three) and tracheitis (two of five). Bacteria were not visualized in these organs by Gram's stain. The other tissues and organs were histologically unremarkable.

Eyes, including periorbital tissues and conjunctivas, taken at necropsy from one adult male pine grosbeak (one eye) and two adult female evening grosbeaks (one eye from one, and both eyes from the other) were frozen and sent on dry ice to the North Carolina State University College of Veterinary Medicine (Raleigh, North Carolina, USA). Upon arrival the tissues were thawed and suspended in 2 ml Frey's broth medium (FMS) supplemented with 15% inactivated swine serum (Kleven, 1998), then vortexed for 1 min, allowed to settle for 5 min and vortexed again for 1 min. The samples were centrifuged at low speed (300 rpm for 1 min) and the media transferred from the tissue debris to sterile tubes and incubated at 37 C. After overnight incubation the broth cultures were centrifuged at 500 rpm to pellet residual debris. The supernatant was passed through a 1.2 μm filter, then divided into two aliquots. One was incubated at 37 C for mycoplasma culture, and the other was used for DNA extraction and *M. gallisepticum*-specific polymerase chain reaction (PCR) as described by Lauerman (1998).

Each of four samples (one from a adult male pine grosbeak and three from two adult female evening grosbeaks) tested by the *M. gallisepticum*-specific PCR was positive (Fig. 3). These PCR results indicated that *M. gallisepticum* DNA was present in the periorbital tissues submitted. Mycoplasma broth cultures were passaged periodically to fresh FMS agar media. After 5 wk of incubation, colonies morphologically consistent with mycoplasmas were isolated on FMS agar only from the female evening grosbeak that had both eyes submitted for culture. Species identification of mycoplasmal colonies on FMS agar was performed by direct immunofluorescence using fluorescein-conjugated rabbit antisera provided by S. H. Kleven (Department of Avian Medicine, University of Georgia, Athens, Georgia, USA).

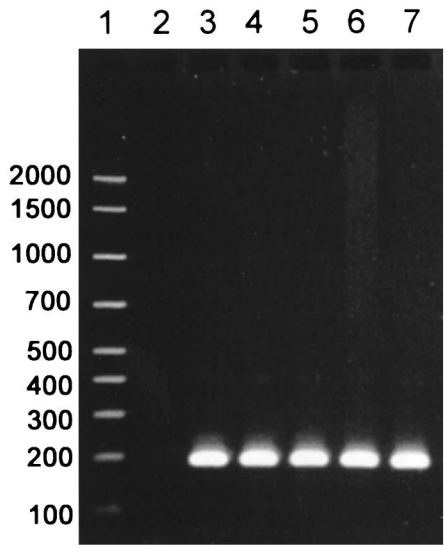

FIGURE 3. *M. gallisepticum*-specific polymerase chain reaction products from periocular tissues. Lane 1: DNA bp ladder (2000, 1500, 1000, 700, 500, 400, 300, 200, 100); lane 2: negative control; lane 3: positive control (*M. gallisepticum* R strain); lane 4: pine grosbeak, adult male, one eye; lane 5: evening grosbeak, adult female, one eye; lanes 6 and 7: evening grosbeak, adult female, left and right eyes respectively.

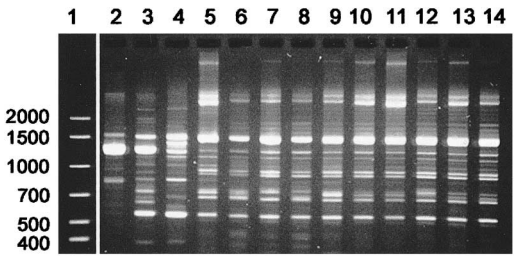

FIGURE 4. Random amplified polymorphic DNA (RAPD) fingerprints of *M. gallisepticum* vaccine strains (lanes 2–4), and house finch (lanes 5–12) and evening grosbeak (lanes 13–14) isolates. Lane 1: DNA bp ladder (2000, 1500, 1000, 700, 500, 400); lane 2: F strain vaccine; lane 3: ts-11 strain vaccine; lane 4: 6/85 strain vaccine; lane 5: North Carolina house finch; lane 6: Maryland house finch; lane 7: Pennsylvania house finch; lane 8: Tennessee house finch; lane 9: Kentucky house finch; lane 10: Michigan house finch; lane 11: Georgia house finch; lane 12: Ohio house finch; lanes 13 and 14: Canada evening grosbeak (left eye and right eye, respectively).

Mycoplasmal colonies isolated from both eyes of this bird fluoresced when tested with the *M. gallisepticum* conjugate. It is likely that *M. gallisepticum* isolates were not made from the other samples submitted due to loss of viability prior to culture, which would not have affected the ability of PCR to detect *M. gallisepticum* DNA.

Mycoplasma gallisepticum isolates from the female evening grosbeak were analyzed by random amplified polymorphic DNA (RAPD) fingerprinting as previously described (Ley et al., 1997) using methods modified from Geary et al. (1994). The RAPD banding pattern for these two isolates was essentially identical to each other and to those isolated from house finches with conjunctivitis from multiple locations in the USA (Fig. 4). As reported previously (Ley et al., 1997) isolates of the songbird strain of *M. gallisepticum* showed RAPD banding patterns (Fig. 4) that differed from *M. gallisepticum* vaccine strains (F, ts-11, and 6/85) used in domestic poultry.

Specimens sampled and submitted for routine bacteriology culture included conjunctiva (five evening grosbeaks and two pine grosbeaks), lung (three evening grosbeaks and two pine grosbeaks), and liver (one evening grosbeak). The specimens were cultured on 5% sheep blood agar (Columbia blood agar base; Difco, Detroit, Michigan, USA). Plates were incubated at 37 C for 4 to 7 days in 5% CO_2 atmosphere. All cultures were negative for specific pathogenic organisms.

This is the first report of *M. gallisepticum* identification in pine grosbeaks and evening grosbeaks with ocular and upper respiratory tract inflammation. Macroscopic and microscopic lesions in the grosbeaks examined were identical to those reported in house finches and American goldfinches infected with *M. gallisepticum* (Ley et al., 1996; Luttrell et al., 1996, 1998). In addition, RAPD fingerprints of *M. gallisepticum* isolates from the evening grosbeak were essentially identical to those of house finches and American goldfinches reported previously (Ley et al., 1997). Taken together, these observations suggest that the finch strain of *M. gallisepticum* is causally implicated in this recent outbreak of con-

junctivitis in pine grosbeaks and evening grosbeaks in Quebec.

Interestingly, *M. gallisepticum* infection in wild birds has been diagnosed only in species from the family Fringillidae (Fischer et al., 1997). The social behavior of fringillids at feeders, where they constitute large flocks, may contribute to the transmission of the infection. It is also possible that this family of birds is more susceptible to *M. gallisepticum* infection than other families of birds.

The effects of *M. gallisepticum* on pine grosbeak and evening grosbeak populations are difficult to estimate. The consequences of mycoplasmosis on grosbeak populations remains undetermined, even though individual birds with conjunctivitis often appeared debilitated. Persistence of the finch strain of *M. gallisepticum* in grosbeak populations is yet to be determined: the occurrence of grosbeaks with conjunctivitis in 2000 suggests that mycoplasmosis may be enzootic in these species. This observation needs to be confirmed by identification of *M. gallisepticum* in additional cases.

From this report and previous studies (Porter, 1994; Fischer et al., 1997; Ley et al., 1997; Hartup et al., 2000), at least five species of fringillid birds have experienced conjunctivitis associated with a single strain of *M. gallisepticum.* Some of these bird species may now constitute reservoirs for this pathogen. The potential for transmission of this *M. gallisepticum* strain to domestic poultry seems limited (Stallknecht et al., 1998). However, the potential for transmission of this organism to other wild bird species remains undetermined.

We are grateful to J. Cardin, J. Deslandes, M. Langlois, L. Pépin, and B. Pépin-Faille for skilful laboratory assistance. This study was funded by the Canadian Cooperative Wildlife Health Centre and the Centre Québécois sur la Santé des Animaux Sauvages.

LITERATURE CITED

DHONDT, A. A., D. L. TESSAGLIA, AND R. L. SLOTHOWER. 1998. Epidemic mycoplasmal conjunctivitis in house finches from eastern North America. Journal of Wildlife Diseases 34: 265–280.

FISCHER, J. R., D. E. STALLKNECHT, M. P. LUTTRELL, A. A. DHONDT, AND K. A. CONVERSE. 1997. Mycoplasmal conjunctivitis in wild songbirds: The spread of a new contagious disease in a mobile host population. Emerging Infectious Diseases 3: 69–72.

GEARY, S. J., M. H. FORSYTH, S. ABOUL SAOUD, G. WANG, D. E. BERG, AND C. M. BERG. 1994. *Mycoplasma gallisepticum* strain differentiation by arbitrary primer PCR (RAPD) fingerprinting. Molecular Cell Probes 8: 311–316.

HARTUP, B. K., G. V. KOLLIAS, AND D. H. LEY. 2000. Mycoplasmal conjunctivitis in songbirds from New York. Journal of Wildlife Diseases 36: 257–264.

KLEVEN, S. H. 1998. Mycoplasmosis. *In* A Laboratory manual for the isolation and identification of avian pathogens, 4th Edition, D. E. Swayne, J. R. Glisson, M. W. Jackwood, J. E. Pearson, and W. M. Reeds (eds.). American Association of Avian Pathologists, Kennett Square, Pennsylvania, pp. 74–80.

LAUERMAN, L. H. 1998. Mycoplasma PCR Assays. *In* Nucleic acid amplification assays for diagnosis of animal diseases, L. H. Lauerman (ed.). American Association of Veterinary Laboratory Diagnosticians. Turlock, California, pp. 41–42.

LEY, D. H., J. E. BERKHOFF, AND J. M. MCLAREN. 1996. *Mycoplasma gallisepticum* isolated from house finches (*Carpodacus mexicanus*) with conjunctivitis. Avian Diseases 40: 480–483.

———, ———, AND S. LEVISOHN. 1997. Molecular epidemiological investigations of *Mycoplasma gallisepticum* conjunctivitis in songbirds by random amplified polymorphic DNA analyses. Emerging Infectious Diseases 3: 375–380.

LUTTRELL, M. P., J. R. FISCHER, D. E. STALLKNECHT, AND S. H. KLEVEN. 1996. Field investigation of *Mycoplasma gallisepticum* infections in house finches (*Carpodacus mexicanus*) from Maryland and Georgia. Avian Diseases 40: 335–341.

———, D. E. STALLKNECHT, J. R. FISCHER, C. T. SEWELL, AND S. H. KLEVEN. 1998. Natural *Mycoplasma gallisepticum* infection in a captive flock of house finches. Journal of Wildlife Diseases 34: 289–296.

PORTER, S. 1994. Conjunctivitis in finches. The National Wildlife Rehabilitators Association Quarterly Winter 1994: 11.

STALLKNECHT, D. E., M. P. LUTTRELL, J. R. FISCHER, AND S. H. KLEVEN. 1998. Potential for transmission of the finch strain of *Mycoplasma gallisepticum* between house finches and chickens. Avian Diseases 42: 352–358.

Received for publication 15 July 2000.