Journal of Avian Medicine and Surgery 11(1):20–24, 1997

Evaluation of Treatment of Conjunctivitis Associated with *Mycoplasma gallisepticum* in House Finches (*Carpodacus mexicanus*)

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Abstract: Thirty-seven house finches (*Carpodacus mexicanus*) with conjunctivitis were admitted to a rehabilitation center. Six (35%) of 17 conjunctival swab samples collected before treatment began were positive on culture for *Mycoplasma gallisepticum*. All birds were treated with ciprofloxacin hydrochloride ophthalmic solution (one drop in each eye [OU] q12h) for 5 to 7 days and tylosin tartrate in water (1 mg/ml) as the sole source of drinking water for 21 to 77 days. Conjunctivitis was resolved in all birds after therapy was completed (60 to 177 days). Conjunctival swab samples from all birds collected after treatment and submitted for culture were negative for *M. gallisepticum*. Two (11%) of the 18 conjunctival swab samples collected after treatment were positive for *M. gallisepticum* by polymerase chain reaction (PCR). Serum samples from 2 (5.4%) other of the 37 birds were antibody positive for *M. gallisepticum* by serum plate agglutination; serum samples from these two birds were antibody negative when retested 22 days later. No gross lesions were found at necropsy in 16 house finches that had been treated, including the birds that tested positive by PCR or serum plate agglutination. Tissue samples of conjunctiva, choanae, infraorbital sinus, trachea, lungs, air sacs, and reproductive organs collected at necropsy were negative for mycoplasma by culture (n = 16) and PCR (n = 15). We did not find evidence of *M. gallisepticum* infection in house finches after treatment with tylosin tartrate administered orally and ciprofloxacin hydrochloride ophthalmic solution applied topically.

Key words: Mycoplasma gallisepticum, conjunctivitis, treatment, house finch, *Carpodacus mexicanus*

Introduction

An epizootic of conjunctivitis in house finches (*Carpodacus mexicanus*) associated with *Mycoplasma gallisepticum* infection was reported from several mid-Atlantic and eastern states in 1994 and 1995.[1,2] The clinical signs and gross lesions ranged from mild to severe unilateral or bilateral conjunctival swelling with serous to mucopurulent drainage and nasal exudate.[2] Isolation of *M. gallisepticum* from house finches admitted to rehabilitation centers[2] raised concern about the potential spread of the infection into free-ranging birds and poultry through the release of rehabilitated birds.

Mycoplasma gallisepticum is the etiogenic agent of chronic respiratory disease in chickens and infectious sinusitis in domestic and wild turkeys, diseases that are economically important to the poultry industry.[3] It has not been proved that passerine birds are a source of naturally occurring outbreaks in poultry.[4,5] However, the isolation of *M. gallispeticum* from previously mycoplasma-free chickens housed in the same enclosure as experimentally inoculated house sparrows documents the potential for transmission between passerine birds and poultry.[6] Additionally, the isolation of *M. gallisepticum* from a blue jay (*Cyanocitta cristata*) that developed conjunctivitis after being housed in cages previously occupied by affected house finches suggests that transmission can occur between passerine species.[2]

Poultry are considered to be persistent carriers of

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M. gallisepticum once infected, even after antibiotic therapy.[3] If this also occurs in house finches, release of treated birds to the wild would be inadvisable. However, *M. gallisepticum*-infected house finches are treated at rehabilitation centers with the expectation of eventual release.[7] Therefore, the effectiveness of a therapeutic protocol in eliminating infection from affected house finches must be examined. In this report we describe the treatment of birds with conjunctivitis associated with *M. gallisepticum* at a rehabilitation center and the evaluation of the birds after treatment.

Materials and Methods

Birds and treatment protocol

Thirty-seven house finches were admitted to Tri-State Bird Rescue and Research (TSBRR, Newark, DE, USA), between 25 August 1994 and 10 February 1995 from Delaware, Maryland, and Pennsylvania. All birds had clinical signs of conjunctivitis (unilateral or bilateral swelling of the facial skin and eyelids, lacrimation, congestion of conjunctival vessels, and periocular swelling). Six birds also presented with or developed proliferative, encrusted nodules on the face or legs, suggestive of avian pox. Conjunctival swab samples were collected for mycoplasmal culture with sterile dacron-tipped swabs (Calgi-Swab, Spectrum Laboratories, Houston, TX, USA) from 17 birds before treatment began.

Initially, the affected eyes of all birds were treated with saline flushes and ciprofloxacin hydrochloride ophthalmic solution (one drop each eye [OU] q12h) (Ciloxan, Alcon Laboratories, Fort Worth, TX, USA) for 5 to 7 days. When ciprofloxacin hydrochloride ophthalmic solution was unavailable, polymyxin B sulfate/neomycin sulfate/gramicidin ophthalmic solution was substituted. Eighteen birds received dexamethasone sodium phosphate ophthalmic solution (one drop OU q12h) for 1 to 2 days to reduce blepheroedema. Presumptive pox lesions on the face or legs in six birds were treated with tincture of iodine applied topically to the skin lesions q12h for 7 to 14 days. Additional medications administered after clinical assessment included one dose of 2.5% dextrose in lactated Ringer's solution (0.5–1 ml SC) (n = 8), amoxicillin trihydrate and clavulanate potassium (3.5 mg PO q12h)(Clavamox Drops, SmithKline Beecham Animal Health, West Chester, PA, USA) for 6 to 7 days (n = 2), naphazoline hydrochloride ophthalmic solution (one drop OU q12h) for 3 days (n = 1), and one dose of dexamethasone (0.04 mg IM) (n = 1).

The first isolation of *M. gallisepticum* from house finches was reported on 13 October 1994. At that time, all 32 of the birds previously admitted with clinical signs of disease were treated with tylosin tartrate (Tylan, Eli Lilly and Company, Indianapolis, IN, USA) in water (4 g/3.8 L [approximately 1 mg/ml]) as the sole source of drinking water for 21 to 77 days. When individual birds were added to a group being treated in a single cage, the duration of treatment was extended by 21 days beyond the addition of the last bird.

The five birds admitted after 13 October 1994 received ophthalmic medications simultaneously with the tylosin administered in the drinking water. Because of the concern about releasing potential asymptomatic carriers, the 37 birds were transported to the North Carolina State University College of Veterinary Medicine (NCSU, Raleigh, NC, USA) for evaluation and maintained in isolation (biosafety level 2) facilities.

Posttreatment protocol

After a 21-day acclimation period, all 37 birds were examined for signs of conjunctivitis, and conjunctival swab samples were taken for mycoplasmal culture 38 to 155 days after the end of treatment. Eighteen of the conjunctival swab samples were also evaluated for *M. gallisepticum* by polymerase chain reaction (PCR). A 0.2-ml blood sample was taken from the right jugular vein of each bird to test for antibodies to *M. gallisepticum* by serum plate agglutination; the sera were separated, then stored at 4°C until testing. The sera from three birds clotted, and repeat samples were taken.

Necropsies were done on 16 birds to collect tissue samples from internal organs. The 16 birds included 15 birds that were euthanatized with carbon dioxide and one bird that died after blood collection. Four birds that were euthanatized had tested positive for *M. gallisepticum* by PCR (n = 2) or serum plate agglutination (n = 2), and 11 birds were selected arbitrarily. The 21 remaining live birds were returned to TSBRR.

Immediately before euthanasia, blood samples were collected for repeat evaluation by serum plate agglutination; the sera from two birds clotted and could not be evaluated. Three pools of samples were collected for mycoplasmal culture from each bird at necropsy: swab samples of conjunctiva, choanae, and infraorbital sinuses; swab samples of lung parenchyma and interclavicular and abdominal (bilateral) air sacs, and 5-mm sections of proximal and distal trachea; and both testes or the entire ovary and oviduct (when visible). Samples from all 15

birds that were euthanatized were evaluated for *M. gallisepticum* by PCR.

Culture methods

Swab specimens were immediately inoculated into 2 ml of Frey's broth medium[8] supplemented with nicotinamide adenine dinucleotide–cysteine and 15% swine serum (FMS); tissue specimens were placed directly into the medium. Samples collected before treatment at TSBRR were stored at 4°C and sent overnight to NCSU. Samples collected at NCSU were stored at 4°C for less than an hour. The broth cultures were incubated at 37°C in humidified air and examined daily for mycoplasmal growth, indicated by a color change of the medium or a visible swirl of turbidity on agitation. A 0.2-ml sample of broth culture was passaged to 1.8 ml of fresh FMS broth and plates of FMS agar media[9] when mycoplasmal growth was indicated, or at 7- to 10-day intervals. Plates were incubated as above and examined daily for colonies of mycoplasma. Primary broth cultures and passages to fresh FMS broth and agar media were incubated until mycoplasma were identified on agar medium, or for up to 40 days from inoculation. Mycoplasma species in colonies on agar plates were determined by direct immunofluorescence[10,11] with fluorescein-conjugated rabbit antisera to *M. gallispeticum*. The antisera were provided by S. Kleven (Department of Avian Medicine, College of Veterinary Medicine, The University of Georgia, Athens, GA, USA).

Serum plate agglutination

Serum plate agglutination testing was conducted with commercially prepared *M. gallisepticum* antigen S-6 serotype (Nobilis, Intervet, Millsboro, DE, USA) according to the manufacturer's instructions, with the exception that 30 μl each, rather than 50 μl, of sample sera and antigen were used. Sample serum and antigen were mixed, rotated for 5 seconds, incubated for 55 seconds, rotated again for 5 seconds, and read 55 seconds later. A positive reaction was characterized by particle agglutination. Positive (National Veterinary Services Laboratory, Ames, IA, USA) and negative (*M. gallisepticum*-free chickens) control sera were used for all testing.

Polymerase chain reaction

Eighteen of the FMS broths inoculated with conjunctival swab samples were evaluated by PCR. A composite of the FMS broths inoculated with tissue samples collected at necropsy was made and evaluated by PCR. A nonradioactive probe-based test (FlockCheck PROBE *Mycoplasma gallisepticum* DNA Test Kit, IDEXX Laboratories, Westbrook, ME, USA) with PCR amplification for the specific detection of *M. gallisepticum* genomic DNA was performed according to the manufacturer's instructions. Briefly, a 1-ml aliquot of each sample broth was transferred to a 1.5-ml conical screw-capped microcentrifuge tube (Fisher Scientific Company, Pittsburg, PA, USA) and centrifuged for 10 minutes at 16,000 × *g* in a microcentrifuge. The supernatant of each tube was discarded, the pellets were washed with a 1-ml sample wash buffer (IDEXX Laboratories) and centrifuged as above, and then the wash step was repeated. Amplification of DNA by PCR was performed for 4 to 4.5 hours in a thermal cycler (Perkin Elmer Corporation, Norwalk, CT, USA). Detection of DNA was performed by pipetting 1.5 μl of each PCR amplificate onto a nylon membrane. Samples were fixed in 0.4 N NaOH; membranes were blocked, and samples were then reacted with enzyme-conjugated probes, washed, and developed with substrate. Developed membranes were rinsed with 1% glacial acetic acid and dried on absorbent paper.

Results

Conjunctival swab samples collected before treatment from 6 (35%) of the 17 house finches were positive for *M. gallisepticum* on culture. Conjunctivitis recurred 6 to 32 days after therapy was discontinued in 11 (34%) of the 32 birds that were treated with ophthalmic antibiotics alone before tylosin was added to the treatment protocol. Eye lesions usually resolved within 13 days (7 to 20 days) after treatment with tylosin began.

All 37 house finches remained free of conjunctivitis for 60 to 177 days after completion of tylosin treatment. No gross lesions were present in any of the 16 birds that were necropsied. *Mycoplasma gallisepticum* were not cultured from any of the antemortem (n = 37) or postmortem (n = 16) samples collected from the birds after treatment.

Two (5.4%) of the 37 house finches tested positive for antibodies to *M. gallisepticum* by serum plate agglutination after treatment. Both birds were among those that had positive culture results before treatment. Samples from all 13 birds that were retested 22 days later by serum plate agglutination were negative, including those from the two birds that previously tested positive.

Conjunctival swab samples collected after treatment from 2 (11%) of 18 house finches were positive by PCR. A pretreatment culture was negative in one of the two birds, and a pretreatment culture

was not done in the second bird. All 15 postmortem samples that were tested by PCR were negative, including those from these two birds.

Discussion

Mycoplasma gallisepticum is the likely etiogenic agent of the epizootic of conjunctivitis seen in free-ranging house finches.[1,2] Positive cultures before treatment demonstrated the presence of *M. gallisepticum* in the birds in this study. We presumed that *M. gallisepticum* also caused the conjunctivitis in those birds that had negative results and those that were not cultured before treatment. The contribution of poxviruses to the morbidity of these finches was not examined.

Clinical signs of conjunctivitis associated with *M. gallisepticum* resolved after oral administration of tylosin and topical application of ophthalmic antibiotics. No recurrence of clinical signs occurred after the combination therapy was discontinued, and *M. gallisepticum* was not isolated from any of the birds after treatment. The recurrence of clinical signs after treatment with ophthalmic drugs alone suggests that this treatment was not sufficient. The efficacy of tylosin administered in the drinking water as a single therapeutic agent could not be evaluated, as tylosin was not administered as the sole medication to any bird.

Therapy was not withheld from any of the birds to determine the natural course of *M. gallisepticum* infection in house finches. However, the variation in the severity of clinical signs at initial presentation suggests that a progression from mild to severe disease occurs in the wild. If the therapy had no effect on the disease outcome, we would have expected clinical signs in some of the birds that were mildly affected to worsen despite treatment.

Treatment of *M. gallisepticum* in passerine birds has not been described, and it is not known if chronic carriers of *M. gallisepticum* exist among these species. The course of *M. gallisepticum* infection in poultry is prolonged and often results in persistent carriers, even after antibiotic treatment.[3] *Mycoplasma gallisepticum* has been isolated from infected chickens treated with tylosin at 500 μg/ml of water offered as the sole source of drinking water for 4 to 8 days.[12] The house finches in this study were offered water with twice that concentration of tylosin for a minimum of 13 days longer than the treatment period in poultry. The drug concentration for the house finches was derived empirically in an effort to adjust for the higher metabolic rate of passerine birds relative to chickens.[13] The minimum length of therapy was determined by exceeding the time in which all birds no longer exhibited clinically apparent eye lesions. The large variability in the lengths of treatment was because of space limitations that resulted in newly acquired birds being placed in cages with birds already being treated. No birds were treated for less than 21 days, therefore we could not determine if shorter lengths of therapy are effective.

Free-ranging nongallinaceous birds have been sampled for *M. gallisepticum* because of concern about transmission to poultry. *Mycoplasma gallisepticum* was isolated from house sparrows (*Passer domesticus*) and doves (*Streptopelia* sp) in India[14] and from tree sparrows (*Passer montanus*) in Japan.[15] Both studies were of birds that were associated with poultry infected with *M. gallisepticum*; clinical signs were not reported in the nondomestic species. *Mycoplasma gallisepticum* was not recovered in free-ranging birds in Spain,[4] nor in wild birds collected on farms housing *M. gallisepticum*-infected turkeys in the USA.[5] Isolation of *M. gallisepticum* in wild passerine birds free of poultry contact has not been reported previous to the current epizootic in house finches.

The significance of the serum plate agglutination test results obtained in this study is unclear, because the specificity and sensitivity of the test in house finches are not known. Serum plate agglutination test results in experimentally infected house sparrows have not correlated with *M. gallisepticum* culture results, but the birds considered as controls may have had contact with infected birds.[6] Positive test results have been reported for *M. gallisepticum* culture-negative passerine birds captured on farms with *M. gallisepticum*-infected turkeys.[4] Because gallinaceous birds infected with *M. gallisepticum* become chronic carriers and remain positive on serum plate agglutination testing after infection,[16,17] it is not known if antibody levels decline after the organism is eliminated. Such a decline apparently occurs in house finches. The antibodies specific to *M. gallisepticum* that were present at initial sample collection in this study decreased to undetectable levels by the time of retesting 22 days later, presumably after *M. gallisepticum* was eliminated.

The positive PCR results and negative culture results in antemortem testing of two birds in this study may reflect the presence of DNA specific to *M. gallisepticum* in the absence of viable organisms. Also, false positive test results are possible. Results were negative when these two birds were retested by PCR 22 days later. The change in PCR test results may indicate that *M. gallisepticum* DNA was cleared after infection was resolved. Alternately, the change may reflect a dilution effect, because the

broths of the various tissue samples were pooled for the second sample.

Reports of assessment of rehabilitated animals for infectious agents before release are rare, despite the potential risks of disseminating diseases.[18,19] To determine the degree of risk associated with the release of house finches infected with *M. gallisepticum* to the wild, basic epidemiologic information such as prevalence, incidence, species susceptibility, and mode of transmission are required. In our study we did not find evidence of *M. gallisepticum* infection in house finches after combination treatment with tylosin tartrate (1 mg/ml of water) as the sole source of drinking water for at least 21 days and ciprofloxacin hydrochloride ophthalmic solution applied topically for 5 to 7 days. We caution that the presence of a *M. gallisepticum* carrier state in treated house finches cannot be ruled out based on the results of this study.

Acknowledgments: We thank Julie E. Mashima, Karen Beck, Patricia Dennis, and Douglas E. Anderson for their valuable technical assistance. Resources to support this research were provided by the state of North Carolina.

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