DIAGNOSIS AND TREATMENT OF CONJUNCTIVITIS IN HOUSE FINCHES ASSOCIATED WITH MYCOPLASMOSIS IN MINNESOTA

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ABSTRACT: An ongoing outbreak of *Mycoplasma gallisepticum*-associated conjunctivitis in house finches (*Carpodacus mexicanus*) that began in 1994 in the eastern United States has been spreading westward. House finches presenting with the clinical signs of *M. gallisepticum*-associated conjunctivitis were first seen at the Wildlife Rehabilitation Center of Minnesota (Minnesota, USA) in July of 1996, and 42 cases were admitted from 26 December 1996 to 10 August 1997. A nested PCR was designed for sensitive and specific detection of the presence of the organism. Twelve birds were treated with oral enrofloxacin (15 mg/kg, twice daily for 21 days) and ophthalmic gentamicin (twice daily for 21 days). All treated birds showed resolution of clinical signs. Following treatment, finches were held for up to 6 mo and tested for the presence of *M. gallisepticum* by culture and nested polymerase chain reaction (PCR). Eight of twelve finches (67%) were positive for *M. gallisepticum* by nested-PCR and four (33%) were positive by culture. The results suggest that oral enrofloxacin and opthalmic gentamicin are not an effective treatment for the eradication of *M. gallisepticum* in house finches. Further, the results show that nested PCR is an effective method for detection of *M. gallisepticum* in house finches and was more sensitive than culture.

Key words: *Carpodacus mexicanus,* enrofloxacin, epizootic, fluoroquinolone, house finch, *Mycoplasma gallisepticum,* nested-polymerase chain reaction.

INTRODUCTION

The house finch (*Carpodacus mexicanus*) population in eastern North America is thought to have arisen from a small number of birds released in the New York City area in the 1940s (Veit and Lewis, 1996). House finches were first sighted in Minnesota (USA) in 1980 (Janssen, 1987).

House finches with conjunctivitis and impaired vision initially were observed at backyard bird feeders in suburban Washington, D.C. (USA) in February 1994 (Fischer et al., 1997). *Mycoplasma gallisepticum* was isolated from affected finches (Ley et al., 1996. Luttrell et al., 1996). Koch's postulates were fulfilled by reproduction of disease following inoculation of unaffected house finches with a finch-derived *M. gallisepticum* isolate (Fischer et al., 1997). *Mycoplasma gallisepticum* strains also have been isolated from American goldfinches (*Carduelis tristis*) and a Blue Jay (*Cyanocitta cristata*) with con-

junctivitis (Ley et al., 1997). To date, all passerine *M. gallisepticum* isolates are indistinguishable by random amplification of polymorphic DNA (RAPD) (Ley et al., 1997), and arbitrary primer polymerase chain reaction (PCR) (Luttrell et al., 1998), suggesting a recent single source outbreak. The *M. gallisepticum* outbreak has spread rapidly westward and was first seen at the Wildlife Rehabilitation Center of Minnesota (WRC; College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota) in July of 1996.

Mycoplasmosis ranks among the most prevalent and costly infectious diseases of birds worldwide (Ley and Yoder, 1997). In poultry, *M. gallisepticum* causes coryza, sneezing, moist rales, and sinusitis, and impairs reproductive performance. Routes of transmission include bird-to bird, egg-borne, wind-borne, and venereal (Jordan, 1996). There are no clinical signs or lesions that are pathognomonic for any of

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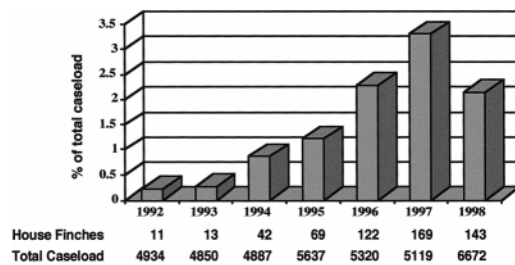|	1992	1993	1994	1995	1996	1997	1998
House Finches	11	13	42	69	122	169	143
Total Caseload	4934	4850	4887	5637	5320	5119	6672

FIGURE 1. House finches as a percentage of Wildlife Rehabilitation Clinic caseload from 1992-98.

the avian mycoplasmas. Therefore, sensitive and specific diagnosis of infected animals is essential to the formulation of rational and effective strategies for treatment and disease control.

The objectives of this study were to describe the outbreak of *M. gallisepticum* in house finches admitted to the WRC, to evaluate the utility of a nested PCR for detection of *M. gallisepticum* in house finches, and to evaluate the efficacy of oral enrofloxacin and ophthalmic gentamicin for treatment of *M. gallisepticum* in house finches.

MATERIALS AND METHODS

The records of the WRC were examined for annual total caseload and annual house finch caseload from 1992 to 1998. The percentage of annual caseload was calculated and analyzed by quadratic linear regression (Weisberg, 1985) in Xlisp-Stat (2.1 Release 3 Beta Release, 1997, University of Minnesota, St. Paul, Minnesota, USA). Finch caseload is presented as a percentage because the overall WRC caseload increased markedly during this period. The percentage of the total caseload at the WRC composed of house finches increased every year from 0.2% in 1992 to 3.3% in 1997 (Fig. 1) as the house finch population established itself in Minnesota. This was followed in 1998 by a drop to 2.1%, which was statistically significant ($P = 0.04$).

From 26 December 1996 to 10 August 1997, 42 house finches were admitted to the WRC with signs of bilateral conjunctivitis. Birds were assessed upon admission, and finches too debilitated for treatment were euthanized by intravenous injection with pentobarbitol (Beuthanasia, Schering-Plough, Kenilworth, New Jersey, USA).

Choanal cleft and tracheal swabs (Calgiswab, Spectrum Laboratories, Houston, Texas,

USA) collected from finches were pre-moistened in Frey's medium (Whitford et al., 1994) and cultured in a shaker incubator at 37 C. Cultures showing a phenol red indicated pH change were filtered through a 0.45 μm filter (Gelman Sciences, Ann Arbor, Michigan, USA) into fresh Frey's media.

Twelve finches were treated with oral enrofloxacin (15 mg/kg, twice daily × 21 days) (Baytril, Bayer, Shawnee Mission, Kansas, USA) and ophthalmic gentamicin (twice daily for 21 days) (Gentocin, Schering-Plough, Kenilworth, New Jersey). To assure consistent dosing, medication was administered directly to each bird. In order to avoid the muscle damage caused by repeated intramuscular injections, which could interfere with releasability, we decided to use an oral route. Enrofloxacin has been shown to have an oral bioavailability of 64% in chickens at 10 mg/kg (Anadon et al., 1995), 48% in African grey parrots at 15 mg/kg (Flammer et al., 1991), and 63% in bustards at 10 mg/kg (Bailey et al., 1998). To our knowledge, there are no published reports of oral bioavailability of enrofloxacin in passerines. Following treatment, finches were held for up to 6 mo and tested for the presence of *M. gallisepticum* by culture and PCR.

All *M. gallisepticum* isolates from wild birds were collected at the WRC. For comparative analysis, we used *M. sturni* isolated from an American crow (*Corvus brachyrhynchos*) and *M. gallopavonis* isolated from a wild turkey (*Meleagris gallopavo*) at the WRC. *Mycoplasma synoviae, M. meleagridis,* and *M. iowae* isolates were graciously provided by S. Kleven (University of Georgia, Athens, Georgia, USA) Poultry *M. gallisepticum* isolates also were graciously provided by S. Kleven and J. Newman, (University of Minnesota).

16S rRNA genes were amplified from cultures or swabs boiled for ten minutes using oligonucleotide primer 1 corresponding to nucleotides 63–83 of the *M. gallisepticum* 16S rRNA (GenBank accession # L08896) (5'-CRAAYGGGTGAG-TAACACGTA, Y = pyrimidine, R = purine) and primer 2, the reverse complement to nucleotides 508–528 (5'-CGRATAACGCTTGCRWCCTAT, W = A or T). The primers were synthesized at the Advanced Genetic Analysis Center (AGAC, University of Minnesota). The 20 μl reaction mixture contained swab material, PCR buffer (Perkin-Elmer, Branchburg, New Jersey), 5% glycerol, 0.4 μM of each primer, 200 μM of dATP, dCTP, dGTP, and dTTP, and 2.5 U of *AmpliTaq* DNA polymerase. The mixtures were amplified in a thermal cycler (Perkin-Elmer, GeneAmp PCR system 2400) with an initial denaturation at 95 C for 5 min, followed by 40 cycles of denaturation at 94 C for 60 sec; annealing at 56 C for

TABLE 1. House finches admitted to the Wildlife Rehabilitation Center of Minnesota with conjunctivitis from 26 December 1996 to 10 August 1997.

Case #	Admit date	Pre-treatment culture	Resolution	Date	Resolution culture	Resolution PCR
96-5301	12/26/96	+	E[a]	6/29/97	−	−
97-0047	1/24/97	−	E	6/29/97	−	+
97-0048	1/24/97	−	E	6/29/97	−	−
97-0063	2/1/97	−	E	6/29/97	+	+
97-0082	2/8/97	−	E	6/21/97	−	+
97-0132	3/10/97	−	D[b]	6/29/97	+	+
97-0173	3/30/97	+	E	6/29/97	−	+
97-0208	4/5/97	−	E	6/29/97	+	+
97-0276	4/18/97	−	E	6/29/97	+	+
97-0440	4/26/97	+	E	6/29/97	−	+
97-1297	5/24/97	−	E	6/29/97	−	−
97-1684	6/2/97	−	E	6/29/97	−	−

[a] E = Euthanized.
[b] D = Died.

30 sec, DNA extension at 72 C for 30 sec, and a final extension step at 72 C for 7 min. For the second *M. gallisepticum*-specific PCR amplification, 0.5 µl of product from the above reaction was then used in a second PCR primer 2 and primer 3 corresponding to nucleotides 423–445 (5′-CAGTTAGTAGAGTGGAAAGCTAT). The 20 µl reaction mixture contained PCR buffer (Perkin-Elmer), 5% glycerol, 0.4 µM of each primer, 200 µM each of dATP, dCTP, dGTP, and dTTP, and 2.5 U of *AmpliTaq* DNA polymerase. The mixtures were amplified with an initial denaturation at 95 C for 5 min, followed by 35 cycles of denaturation at 94 C for 60 sec; annealing at 56 C for 30 sec, DNA extension at 72 C for 30 sec, and a final extension step at 72 C for 7 min. A 5 µl volume of PCR product was mixed with 2 µl of loading buffer (0.2% Orange G in 50% glycerol) and electrophoresed in a 1% agarose gel with 0.5 µg/ml ethidium bromide. Gels were photographed under ultraviolet light using an Eagle Eye II gel documentation system (Stratagene, La Jolla, California, USA).

The sensitivity of this nested polymerase chain reaction (PCR) was determined by testing serial dilutions of a culture. Frey's medium was inoculated with *M. gallisepticum* strain 97-0173 and cultured in a shaker incubator at 37 C until a color change was detected. To determine the titer of the culture, eight replicates of 10-fold serial dilutions were made into fresh Frey's media in 96-well plates (Costar, Cambridge, Massachusetts, USA) to a volume of 100 µl per well and grown for 22 days at 37 C. Growth was determined by pH change as measured by color change of phenol red in the media to yellow. Serial dilutions of the original culture were tested using the nested PCR method.

To confirm sensitivity testing, dilution of DNA was used. The genome size of *M. gallisepticum* is 1,054 kbp (Gorton et al., 1995), so the weight of one genome is approximately 1.06 × 10^{-9} µg. DNA was prepared using QIAamp tissue kit (Qiagen, Valencia, California). The DNA was resuspended in TE buffer (10 mm Tris HCl, 1 mm EDTA, pH 8.0) and quantified by spectrophotometry as well as comparison to a known standard on agarose gel electrophoresis. Serial dilutions of the DNA preparation with known genome numbers were tested using the nested PCR method. Sequencing reactions of isolates 97-0173, 97-0244, and 97-2638 were identified by 16S rDNA sequencing from bases 103-508 (Genbank accession # 317201) with the Ready Reaction Dye Terminator Kit (Perkin-Elmer), 4 pmol primer, and 100 ng PCR product analyzed on ABI 377 automated DNA sequencers at the AGAC.

RESULTS

Forty-two finches with conjunctivitis were admitted to the wildlife clinic from 12/96–8/97 and *M. gallisepticum* was cultured from nine upon admission. Twelve of the 42 finches received full treatment (Table 1), and 30 were euthanized or died. All treated birds showed resolution of clinical signs after initial treatment. One treated finch (97-0082) redeveloped conjunctivitis >3 mo after treatment and was euthanized. Eight of 12 finches had levels of *M. gallisepticum* detectable by PCR after treatment. Four of 12 had growth in cul-
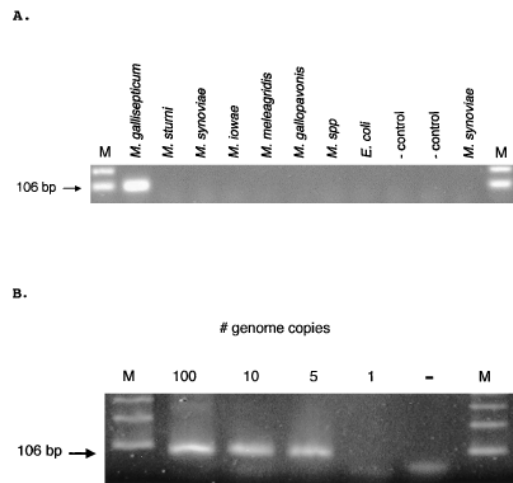
A.

B.

FIGURE 2. Nested PCR in mycoplasmosis of birds. A. Specificity of nested PCR. Products of *M. gallisepticum* nested PCR reactions for different avian Mycoplasma species as templates are presented in lanes 2 through 8. M, 123 bp marker. B. Sensitivity of nested PCR. Products of nested PCR reactions of different dilutions of *M. gallisepticum* are presented in lanes 2 through 8. M, 123 bp marker.

ture after treatment. All treated finches were euthanized by intravenous injection with pentobarbitol (Beuthanasia, Schering-Plough, Kenilworth, New Jersey).

The nested PCR produced a 106 base pair band when *M. gallisepticum* was used as a template. The nested PCR did not amplify samples of *M. synoviae, M. sturni, M. iowae, M. meleagridis, M. gallopavonis,* or a swab from a house finch without conjunctivitis (Fig. 2a). The PCR did amplify *M. gallisepticum* from all thirteen culture-positive birds and three finches with conjunctivitis that were culture-negative as well as the poultry *M. gallisepticum* strains. This nested PCR had a detection limit of one organism using the first method of diluting a culture and 5 genome copies using the second method of diluting DNA (Fig. 2b).

DISCUSSION

Carpodacus mexicanus is a recent arrival to Minnesota, and the representation of house finches in the caseload at the WRC increased rapidly until 1997. The temporal

correlation of detection of *M. gallisepticum* in Minnesota house finches and a significant change in the trend in representation of house finch cases at the WRC suggests that this pathogen may have a significant impact on a wild bird population.

A nested PCR was utilized in this study because it is more sensitive than simple PCR (Miserez et al., 1997). Nested PCR is a rapid, highly sensitive, and specific technique and has several advantages for use in wildlife. Culture of *Mycoplasma* spp. requires specialized media and is slow, requiring up to 4 wk (Jordan, 1996). Polymerase chain reaction has been shown to be a more sensitive test than culture in other *Mycoplasma* spp. (Abele-Horn et al., 1996. Sanchez et al., 1994. Sachse et al., 1993. Tola et al., 1997), and does not require the presence of viable organism. Wildlife samples are often presented postmortem in less than optimal condition and many *Mycoplasma* spp. do not remain viable.

Oral enrofloxacin and ophthalmic gentamicin do not appear to be an effective treatment for the eradication of *M. gallisepticum* in house finches and show that nested PCR is an effective method for detection of *M. gallisepticum* in house finches.

In a previous study (Mashima et al, 1997), 11% of house finches treated with oral tylosin tartrate still carried PCR-detectable levels of *M. gallisepticum* after treatment using the Idexx FlockChek MG DNA Probe, which is capable of detecting 100 organisms from a tracheal swab (Idexx Laboratories, Inc., Westbrook, Maine, USA). In another study, a group of 30 hatchling chickens was infected with a virulent strain of *M. gallisepticum* and treated with tylosin (0.5 g/l). *Mycoplasma gallisepticum* was recovered from five chicks during life and from six dead chicks. (Jordan and Horrocks, 1996). Of even greater concern is the potential release of organisms that have acquired antimicrobial resistance into the environment. One study of 29 poultry *M. gallisepticum* strains from

Japan found that 28% were tylosin-resistant. (Takahashi and Yoshida, 1989). In a study of antimicrobial resistance of *Mycoplasma mycoides* ssp. mycoides strain T1, for which the minimum inhibitory concentration (MIC) of tylosin was less than 0.1 µg/ml, mutants resistant to greater than 100 µg/ml were obtained in three passages (Lee et al., 1987).

Danofloxacin, a fluoroquinolone antimicrobial, has been compared to tylosin at concentrations equivalent for control of mortality and maintenance of weight gain in experimentally infected 1-day-old chicks. It was found that the frequency of reisolation of *M. gallisepticum* from chicks treated with danofloxacin was lower than that from chicks treated with tylosin (Tanner et al., 1993).

Given the above information, the decision to evaluate the efficacy of a fluoroquinolone for the treatment of *M. gallisepticum* in house finches was made, and enrofloxacin was chosen based on availability. Gentamicin was chosen for adjunct therapy because it has activity against *M. gallisepticum* (Lin, 1987), may be synergistic and does not usually interfere with fluoroquinolones (Visalli et al., 1998. Dembry et al., 1997), and was available.

We believe there was persistence of *M. gallisepticum* in house finches throughout the tested regime of treatment with oral enrofloxacin and ophthalmic gentamicin. However, persistence has not been conclusively demonstrated. It is possible that finches were re-infected post-treatment while at the clinic. Potential vectors for re-infection include handlers and fomites. All handlers were instructed to wash their hands before handling other birds, but this may not have been sufficient. Additionally, all finches were housed in the same room, raising the possibility of air-borne fomites. Molecular epidemiological studies using established techniques to determine whether all birds were infected with the same strain are not likely to be helpful, as all reported passerine *M. gallisepticum* isolates are indistinguishable by RAPD (Ley et al., 1997) and AP-PCR (Luttrell et al., 1998). Monitoring and biosecurity practices are advisable to prevent the spread of *M. gallisepticum* and other organisms within rehabilitation facilities.

The goal of wildlife rehabilitation is the release of the animal into the wild. *Mycoplasma gallisepticum* treatment in poultry often results in persistent carriers. Long-term management of individual wild finches is not possible. Given the strong possibility of the existence of a *M. gallisepticum* carrier state in finches, infected birds should not be considered cured after abatement of clinical signs. There are many immunosuppressive factors for birds in wildlife rehabilitation, including the stress of captivity, injury, infection, and glucocorticoid use. The only report of *M. gallisepticum* infection in a Blue Jay occurred in a wildlife rehabilitation setting (Ley et al., 1997). While the decision whether or not to release potential carrier birds is subjective, other sick and injured birds should not be exposed to *M. gallisepticum* infected finches.

ACKNOWLEDGEMENTS

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