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Research Note—

Maintenance of a Captive Flock of House Finches Free of Infection by Mycoplasma gallisepticum

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SUMMARY. Since the beginning of an epidemic of conjunctivitis in wild house finches caused by *Mycoplasma gallisepticum* (MG), all captive colonies established by capturing free-ranging house finches from the eastern population have also either been infected at the time of capture or developed infection shortly after capture. In an attempt to avoid this infection in captive flocks being maintained for studies of the finches' behavior and ecology, we compared two different flock management strategies and were able to prevent the development of mycoplasmal conjunctivitis with one of the strategies. Single-sex flocks were built by introducing only seronegative wild-caught birds showing no clinical signs of conjunctivitis and covering their outdoor flight cages with netting to prevent interaction with other wild birds although only the female flocks were initially treated with a 6-wk course of tylosin tartrate (0.3 mg/ml). The female flocks never developed conjunctivitis although the disease did develop in the male flocks. Furthermore, serologic assessments of the healthy flock by serum plate agglutination assays for MG indicated that the females remained free of MG infection in the final 7 wk of the study, during which they were unmedicated. We conclude that any low-level MG infection not diagnosed by the initial test for seroconversion was cleared by the prolonged drug treatment.

RESUMEN. *Nota de Investigación*—Mantenimiento de una parvada de gorriones en cautiverio libres de la infección por Mycoplasma gallisepticum.

Desde el comienzo de una epidemia de conjuntivitis en un galpón de gorriones silvestres causada por *Mycoplasma gallisepticum*, todas las colonias de gorriones establecidas mediante la captura de grupos de gorriones silvestres de las poblaciones del oriente, habían sido también infectadas al momento de la captura ó desarrollaron infección poco después de la captura. En un intento para evitar esta infección en las parvadas mantenidas en cautiverio para estudios ecológicos y de comportamiento, se compararon dos estrategias diferentes de manejo de la parvada y se pudo prevenir el desarrollo de conjuntivitis causada por *Mycoplasma* con una de las estrategias. Las parvadas de un solo sexo fueron establecidas únicamente a partir de la introducción de aves silvestres capturadas que fueron negativas serológicamente y que no mostraban signos clínicos de conjuntivitis. Además, se cubrió el exterior de las jaula con malla para evitar la interacción con otras aves silvestres. Las hembras recibieron tratamiento durante seis semanas con tartrato de tilosina (0.3 mg/ml). Las parvadas de hembras no desarrollaron conjuntivitis pero la enfermedad sí se desarrolló en las parvadas de machos. Además estudios serológicos de las parvadas mediante la aglutinación en placa para *M. gallisepticum* indicaron que las hembras permanecieron libres de la infección por *M. gallisepticum* hasta el final de la semana séptima del estudio durante la cual no estaban recibiendo tratamiento. Se concluyó que cualquier nivel bajo de infección por *M. gallisepticum* no diagnosticado por medio de la prueba inicial para seroconversión fue eliminado por el prolongado tratamiento con la droga.

Key words: house finch, *Carpodacus mexicanus*, *Mycoplasma gallisepticum*, conjunctivitis, tylosin tartrate, flock management

Abbreviations: MG = *Mycoplasma gallisepticum*; SPA = serum plate agglutination

The house finch (*Carpodacus mexicanus*) is a small granivorous passerine bird native to western North America (5). In the late 1940s an unknown number of house finches from coastal California was released in the vicinity of New York City, and by the late 1980s, this population had expanded to encompass most of the states and provinces east of the Mississippi River (5). Thus, there are both eastern and western populations of house finches. In 1994, house finches were observed in suburban Washington, DC, with a variety of clinical signs, including a pronounced conjunctivitis. *Mycoplasma gallisepticum* (MG), a well-known and widespread pathogen of poultry (16), was isolated from infected finches and confirmed as the etiologic agent, representing the first time MG was observed more than sporadically in birds other than domestic poultry and wild turkeys (9). The disease quickly reached epidemic levels and by 1996 had spread throughout the eastern range of the house finch, although to date it has not spread to the native western population (3). The mortality associated with this infection was high, and by 1997, the eastern population had been reduced by 60% relative to preepidemic levels (2,13). During this period, MG conjunctivitis was observed in all captive flocks of eastern house finches of which we are aware (1,11,15; authors' unpubl. data), confounding the interpretation of data from studies unrelated to the epidemic, such as studies of the finches' behavioral ecology and reproductive biology. Wildlife rehabilitation centers and veterinary clinics also reported dramatic increases in demand for care of sick birds (12), and the commercial poultry industry expressed alarm over the possibility that house finches were potentially a new reservoir for a novel strain of this debilitating pathogen (14). Our purpose in this paper is to report a protocol we employed successfully that allowed us to build and maintain captive flocks of house finches free of MG.

MATERIALS AND METHODS

Capturing birds. All the birds captured as part of this study were intended for use in subsequent experiments, the protocols for which required us to build captive flocks of healthy juvenile birds. Specifically, our subsequent experiments required four flocks of juvenile males and two flocks of juvenile females. During late July and August 1999, we captured 221 juvenile finches with wire mesh basket traps placed around bird feeders on the campus of Auburn University in east-central Alabama. Each bird was placed in its own brown paper bag (13 × 8 × 27 cm), which provided us an inexpensive means of safely isolating individuals while we assessed them and determined whether any given bird would be released or added to one of the captive flocks. We used each bag only once before disposing of it. All adult birds were released, as were all birds presenting clinical signs of mycoplasmal conjunctivitis. After handling any bird with conjunctivitis, we disinfected our hands with a commercially available moist towelette containing an antibacterial agent (Triclosan, 0.2%).

Building flocks. Juvenile house finches can be distinguished from adults by the coloring of their plumage until they have completed their first molt in October or November. In August and September, the molt has generally progressed sufficiently that it is possible to determine the sex of many juvenile birds. All juveniles that we judged to be females were placed in a single cage until it contained approximately 15–20 birds, at which time we began to establish the next flock of females in a new cage. All juvenile males and juveniles of unknown sex were placed into another cage until that also had 10–15 birds, and we began the establishment of another flock of male/unknown sex birds. When any bird of unknown sex molted sufficiently that we could tell it was a female, it was turned loose. Thus, the two flocks of females were built only from birds of known sex, whereas the four male flocks were built from groups that initially contained a handful of individuals later found to be females. This difference in establishment of the flocks is likely minor because in a previous study at the same aviary we found no intersexual differences in susceptibility to MG (authors' unpubl. data).

Serology. We drew 100–200 μl of blood from all of the remaining birds by venipuncture of the brachial vein with a 26.5-gauge needle followed by collection of the blood in heparinized microhematocrit tubes. We separated the plasma from the red blood cells by centrifugation and then tested for MG-specific antibodies by a commercial serum plate agglutination (SPA) assay (InterVet, Millsboro, DE) (10,11; authors' unpubl. data). This assay permits rapid screening of a large number of samples (7). The degree of agglutination of plasma samples was scored on a scale from 0 to 4, with a score of 2 or greater considered positive. To be conservative, we released any bird receiving even a score of 1.

Flock maintenance. Birds were housed in outdoor flight cages (1.5 × 3.7 × 2.4 m) at the Auburn University aviary and were isolated into single-sex flocks. We maintained a total of 50 males in four cages and a total of 36 females in an additional two cages adjacent to one another but not contiguous

with the cages of the males. No cage housed more than 20 birds. All finches received an *ad libitum* diet of birdseed (brown and white millet and black oil sunflower seed) supplemented with grit and with Premium Multi-Drop high potency multivitamins for caged birds (8 in 1 Pet Products, Inc., Hauppage, NY). Water was provided in bowls on a daily basis and contained sulfadimethoxine (0.125 g/liter) to suppress coccidial infection (1).

Differences between male and female flocks. There were two important differences in the way in which the male flocks and female flocks were maintained. First, the cages in which the females were housed had plastic netting over them, separated from the wire mesh of the cage by approximately 25 cm. This netting prevented direct contact between caged birds and wild house finches. This netting is marketed as a nonlethal means to keep wild birds off of ornamental plants and is stiff enough that birds do not become entangled in it. The second, and perhaps more important, difference in housing conditions was that the female finches were maintained continuously on the antibiotic drug tylosin tartrate (0.3 mg/ml added to the drinking water) from their capture on August 24, 1999, until October 4, 1999. This represents a course of treatment lasting six wk. One of the male flocks received only an initial week of treatment and then was unmedicated until clinical signs were noted. The other male flocks received no initial treatment with tylosin until conjunctivitis was noted in the flock. When any bird developed conjunctivitis, it was removed from the flock and placed into a quarantine cage separated from the other flocks in this experiment. The infected bird and the flock from which it had been removed were then started on a course of tylosin tartrate as described above. On November 23, 1999, 7 wk after the females were removed from tylosin treatment, we again bled all birds in the female flocks and tested the plasma for evidence of seroconversion.

RESULTS

Twenty-one (9.5%) of 221 juvenile house finches trapped while building our flocks showed clinical signs of mycoplasmal conjunctivitis and were released. An additional 114 birds were released either because they had SPA scores of ≥ 1 or because we were unable to test them for evidence of seroconversion. The final composition of captive flocks included 36 females in two flocks and 50 males in four flocks.

Individuals from all four flocks of male finches, including the group with a short initial treatment of tylosin, developed mycoplasmal conjunctivitis within 7–10 days after the first bird of the flock was captured. In total, 16 (32%) of the 50 males developed clinical signs of MG infection. This figure probably underrepresents the potential for infection within the male flocks because we isolated all infected individuals when they developed conjunctivitis and concurrently started the male flocks on a course of treatment with tylosin to prevent further spread of the disease. SPA analysis confirmed the presence of antibodies to MG among the infected males.

Even though the females were removed from tylosin treatment in early October, none ever presented with clinical signs during the period of drug treatment or during the 7 wk from the time drug use was discontinued until the study ended. Furthermore, when we bled the two flocks of females in late November, we found no evidence of seroconversion; none of the females showed a SPA score greater than 1. This period of 7 wk without development of clinical signs is two to three times longer than any previous flock has been maintained free of mycoplasmal conjunctivitis and is five times longer than the concurrently built flocks of males remained free of MG (1,11,15; authors' unpubl. data).

Since 1996, there have been 39 separate flocks of house finches maintained at the Auburn University aviary. Except for the two female flocks described in this paper, all birds in previous flocks have been either unmedicated or medicated only briefly with tylosin tartrate, and mycoplasmal conjunctivitis has been observed in every flock. The two seronegative flocks described here were the only ones medicated for a long initial period and excluded from contact with wild birds and were the only ones not to seroconvert or develop clinical signs (chi square = 99.3, df = 3, $P < 0.001$).

DISCUSSION

We were able to prevent a flock of female house finches from developing mycoplasmal conjunctivitis even though the disease developed in other flocks established at the same time with birds taken from the same local wild population. The spread of conjunctivitis in the male flocks in this study was consistent with a pattern of disease seen in every experiment with captive eastern house finches since 1995. The pattern in previous years, including two exper-

imental infections of captive flocks (11; authors' unpubl. data), has been for the initial founding of a seronegative flock with no clinical signs of MG infection to be followed within 1–3 wk by the unanticipated development of clinical signs in at least a handful of individuals. After the first observation of conjunctivitis, the infection typically spreads rapidly through 60%–100% of the flock. Thus, even if only two or three individual females in this study had developed the disease, it would likely have spread through the flock and been detected. The females never developed mycoplasmal conjunctivitis, and evidence from SPA analyses suggests that they never developed more than, at most, a low-level infection of MG.

How, then, did the male flocks become infected, given that they were also seronegative at the time of capture? We see three possibilities. First, it is possible that purely by chance none of the females had been exposed to MG, whereas some of the males had been exposed but had not yet seroconverted at the time of capture. This seems highly unlikely, given that males and females were captured together in the same traps. Twenty-one (9.5%) of the 221 juveniles caught from the wild population of this gregarious species presented with clinical signs of conjunctivitis, so all of the birds we captured seem likely to have encountered infected individuals. Second, it is possible that all the birds were truly uninfected at the time of capture but the males were eventually infected by wild birds landing on their cages and interacting with them. This seems unlikely for the reasons just outlined but also because the opportunity for transmission in these encounters is limited. While Stallknecht *et al.* (14) found that 80% of chickens in direct contact with house finches seroconverted, no transmission was recorded across a wire barrier or across a room. Additionally, the main risk factor identified for transmission of MG in wild house finches was the presence of tube-style feeders shared by both infected and uninfected birds (4). Those birds in our captive flocks that were not isolated by plastic netting still had no opportunity to share food with wild birds that landed on their cages. Indeed, even the netted cages were briefly exposed to wild birds on four occasions when the wild birds managed to find an opening through the netting. Both of our female flocks were exposed to wild birds on those occasions, although a wild bird was inside the netting for less than 1 hr on each occasion. Despite exposure to wild birds, no captive finch in the female flocks seroconverted or developed conjunctivitis, possibly indicating a reduced importance for isolation of captive flocks from wild birds. We cannot conclusively rule out the value of the netting, however, because the four brief instances in which wild birds were inside the netting are in contrast to the extensive, near-daily presence of wild birds on top of the cages containing our male house finches. Finally, we consider it most likely that some proportion of both the males and females chosen for inclusion in our captive flocks were infected with MG but had not yet seroconverted, and the infection was at a level low enough to be eradicated by the prolonged treatment with tylosin tartrate. In a study comparing the efficacies of tilmicosin and tylosin for treatment of artificial MG infection in turkey poults, Jordan *et al.* (6) found treatment with tylosin associated with significantly decreased rates of infection and mortality. However, their protocol involved a dose of tylosin (0.5 mg/ml) significantly higher than that administered here, and they ended their trial after 17 days, limiting the ability to compare our results with theirs. Mashima *et al.* (12) reported resolution of conjunctivitis in house finches treated with tylosin tartrate, supporting the efficacy of that drug in treatment of the disease. However, even though they used a much higher dosage (1 mg/ml) than we report here, they did not address the potential of the drug to prevent initial development of clinical signs, and several of the birds they treated were still positive by either SPA or polymerase chain reaction after resolution of the conjunctivitis.

A previous study (8) suggested that a single strain of MG is responsible for the ongoing epidemic occurrence of mycoplasmal conjunctivitis and found that this strain is distinct from that responsible for mycoplasmosis in poultry. Therefore, the drug treatment we describe here should be effective in preventing infection of captive house finches throughout the eastern portion of their range.

Authors' note. After the end of the experiment described in this paper, all male and female flocks were given a 1-wk prophylactic course of tylosin tartrate (0.3 mg/ml added to

the drinking water), and all flocks were housed in cages covered by netting. Since that time, we have observed no clinical signs of MG in any of our flocks. Thus, as of mid-April 2000, we have maintained flocks of house finches free of mycoplasmal conjunctivitis for 27 wk, representing a period of time 8–10 times longer than any previous flock has been maintained free of MG (1,11,15; authors' unpubl. data).

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