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NATURAL *MYCOPLASMA GALLISEPTICUM* INFECTION IN A CAPTIVE FLOCK OF HOUSE FINCHES

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ABSTRACT: Naturally-occurring mycoplasmal conjunctivitis is described among 104 wild-caught, and initially seronegative, house finches (*Carpodacus mexicanus*) maintained in captivity for 12 wk during November 1995 through January 1996. Finches housed in three pens were monitored for clinical signs, and ≥10 birds were euthanatized for necropsy and mycoplasma testing every 2 wk. Within 2 to 4 wk following initial detection of lesions, >50% of the birds in each of three pens developed a debilitating disease characterized by mild to severe ocular swelling, conjunctivitis, and ocular and nasal discharge. Microscopic lesions in affected finches consisted of mild to severe lymphoplasmacytic inflammation with epithelial and lymphoid hyperplasia in conjunctivae, nasal turbinates, and trachea. *Mycoplasma gallisepticum* infection was confirmed by culture or polymerase chain reaction (PCR) in all birds with conjunctival lesions and in 43% of birds without lesions. An arbitrary primer PCR was used to confirm *M. gallisepticum* isolates as identical to a field strain previously associated with house finch conjunctivitis. Most birds (89%) with conjunctivitis developed a concurrent antibody response detectable by serum plate agglutination (SPA) within 2 wk of lesion development. Hemagglutination inhibition (HI) and enzyme-linked immunosorbent assay (ELISA) tests were less sensitive than the SPA test. The clinical severity of this disease and high proportion of affected birds suggests that *M. gallisepticum* may have a negative impact on free-flying house finch populations.

Key words: *Carpodacus mexicanus*, conjunctivitis, epidemiology, house finch, *Mycoplasma gallisepticum*, serology.

INTRODUCTION

Mycoplasma gallisepticum, an important respiratory pathogen of domestic poultry (Ley and Yoder, 1997), recently has emerged as a disease of house finches (*Carpodacus mexicanus*) in the eastern United States. During the winter of 1994, acute conjunctivitis was reported in house finches associated with bird feeders in the mid-Atlantic states, and *M. gallisepticum* subsequently was cultured from sick finches from Virginia, North Carolina, Delaware (Ley et al., 1996), Maryland, and Georgia (Luttrell et al., 1996). The epornitic has since spread to house finches throughout their entire eastern range (Fischer et al., 1997). The disease is characterized by mild to severe ocular swelling, conjunctivitis, ocular and nasal discharge, and debilitation.

To understand the course of *M. gallisepticum* infection in house finches, we captured a group of finches for experimental inoculation. At the time of capture, birds were clinically normal and seronegative for *M. gallisepticum*. However, after conjunctivitis became apparent in several birds within the first week, we elected to follow the natural course of *M. gallisepticum* through this captive population. Objectives were to evaluate the long-term clinical response, to assess susceptibility, to correlate conjunctival lesions with the presence of *M. gallisepticum*, and to validate existing diagnostic tools for use in this new host.

MATERIALS AND METHODS

House finches were captured by trapping or mist netting at The University of Georgia Veterinary Farm (Clarke County, Georgia, USA; 33°55′N, 83°22′W) in November 1995, under scientific collecting permits from the Georgia Department of Natural Resources (Atlanta, Georgia, USA; No. 29-0000205) and the United States Fish and Wildlife Service (United States Department of the Interior, Atlanta, Georgia, USA; PRT-779238). Captured finches were examined for gross lesions of *M. gallisep-*

ticum, and blood samples were collected in heparinized capillary tubes by brachial venipuncture. Serum was separated by centrifugation and screened by serum plate agglutination (SPA) testing (Glisson et al., 1984) for antibodies to *M. gallisepticum*.

One hundred and four seronegative, clinically normal finches were selected for the study and banded with metal leg or wing bands. Birds were housed in three separate pens (0.6 m × 1.4–1.9 m × 1.4–1.7 m) in the same building at the Poultry Diagnostic and Research Center (PDRC; The University of Georgia, Athens, Georgia, USA) and given mixed seeds (Sunflower, Inc., Granlin, North Dakota, USA) and water ad libitum. Two pens were adjacent, separated by netting (pens A and B), and a third (pen C) was located approximately 3 m across the room. Within the first week of captivity, conjunctivitis was detected in pen C. At this point, five birds with conjunctivitis were bled and cultured, and necropsies were performed to verify the presence of *M. gallisepticum*.

Birds were observed daily and caught weekly for close gross examination. Pens with clinically normal birds were examined first to minimize contamination from handling. Blood samples were collected by brachial venipuncture every 2 wk for 12 wk. Ten birds, five with and five without conjunctival lesions, were euthanatized by cervical dislocation for necropsy at 2, 4, 6, and 8 wk and all remaining birds at 12 wk. Birds were examined for gross lesions, and weights were obtained. Blood samples collected prior to euthanasia by cardiac puncture were centrifuged to separate serum. Conjunctiva, sinus, trachea, and lungs and air sacs from each bird were swabbed separately for mycoplasma culture with Type 1 Calgiswabs (Spectrum Diagnostics, Glenwood, Illinois, USA). Samples of brain, liver, spleen, lung, trachea, heart, kidney, proventriculus, small intestines, skull with nasal turbinates, and eyes with surrounding structures were placed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin for microscopic examination.

If birds became moribund between sampling periods, they were euthanatized and necropsies were performed; the sampling protocol was followed as above for most of these birds. Only tissues for histopathology were collected from birds found dead, because in previous diagnostic cases, culture attempts from dead birds were unsuccessful.

Mycoplasma media included SP4 broth (Whitcomb, 1983) and Frey's media with swine serum agar plates (Kleven and Yoder, 1989). Broth tubes were incubated at 37°C and plated on agar once a week for 4 wk or when a color change occurred. Isolates were identified by fluorescent antibody (FA) testing (Talkington and Kleven, 1983). Aliquots from 24 hr broth cultures were pooled for each bird and tested with a FlockChek MG DNA Probe test kit (IDEXX Laboratories, Westbrook, Maine, USA) as an additional detection method of *M. gallisepticum*. To verify isolates, an arbitrary primer PCR (AP-PCR) (Fan et al., 1995) using primer no. 1254 (Geary et al., 1994) was used to compare isolates made from finches at the beginning and end of the study with isolates from a previous field study (Luttrell et al., 1996) and with several standard poultry strains of *M. gallisepticum*.

Serum samples were tested by SPA and hemagglutination inhibition (HI) tests (Kleven and Yoder, 1989) using laboratory-prepared antigens from the A5969 strain of *M. gallisepticum*. The SPA reactions were scored 0 to 4+ based on the degree of agglutination with ≥2+ as positive and ≤1+ as negative; HI titers ≥1:40 were considered positive. To minimize incompatibility in the HI test, the erythrocyte suspension used in this test was made from red blood cells collected from house finches seronegative for *M. gallisepticum*. Sera collected at necropsy also were tested by a commercial enzyme-linked immunosorbent assay (ELISA) kit (Diagnosticum, Budapest, Hungary) according to the manufacturer's directions.

At 6 and 8 wk birds were tested for other bacteria. Conjunctival swabs were streaked directly onto blood and MacConkey agars according to procedures described for the culture of *Haemophilus paragallinarum* (Blackall and Yamamoto, 1989) and *Bordetella avium* (Arp and Skeeles, 1989). Liver and conjunctival impression smears were made for examination of *Chlamydia* spp. by FA testing (Andersen and Tappe, 1989).

RESULTS

Gross conjunctival lesions were detected in pen C within the first week of captivity and became evident in most birds (76%) in all three pens during the 12 wk-study. Although development of clinical disease in each pen began at different times, most birds were affected within 2 to 4 wk once a few individuals within the pen developed lesions (Fig. 1). Clinical signs included mild to severe eyelid swelling in one or both eyes, watery ocular discharge, and dried nasal exudate (Fig. 2). Coughing, sneezing, and rales were not observed. Some birds that initially had

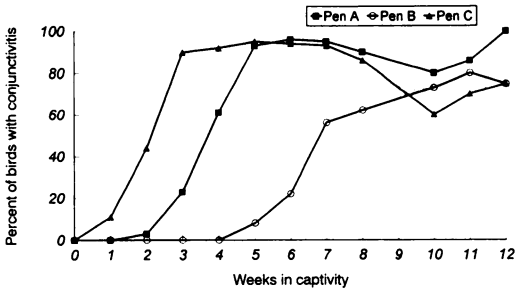

FIGURE 1. Percentage of house finches (*Carpodacus mexicanus*) with conjunctival lesions caused by *Mycoplasma gallisepticum* during a 12 wk captive study.

FIGURE 2. House finch (*Carpodacus mexicanus*) with conjunctivitis caused by *Mycoplasma gallisepticum*. Eyelid swelling, periorbital alopecia, and serous ocular discharge are present, and the nostril is plugged with mucoid exudate.

unilateral lesions eventually developed bilateral lesions, but many birds had only one affected eye. Lesions never completely resolved in any bird, and the degree of severity usually remained constant. For example, birds with mild swelling did not necessarily progress to a more severe stage through time. By 12 wk, only three of 17 remaining birds appeared unaffected.

Necropsies were performed on 38 birds with lesions and 23 birds without lesions at the 2 wk sampling intervals. Although adequate food and water were provided ad libitum, 43 birds either died or became so debilitated that they had to be euthanatized. Conjunctival swelling sometimes progressed to the point that vision was obscured, but even birds with mild swelling often exhibited depression and became dehydrated and weakened. Although birds were not weighed before the study began, weights obtained at necropsy showed a decrease in mean body weight over time in relation to duration of gross lesions. Mean (±SD) weights varied from 19.9 ± 1.92 g in birds without gross lesions (n = 22) to 15.6 ± 1.37 g in birds that had lesions more than 6 wk (n = 14), a difference of 22% (Fig. 3).

Microscopic lesions were limited to ocular tissues, nasal turbinates, and trachea (Table 1). Lung lesions were not apparent, nor were lesions in any of the additional tissues examined. Conjunctival lesions consisted of mild to severe lymphoplasmacytic infiltration of the submucosa and mucosa with epithelial and lymphoid hyperplasia (Fig. 4). Lymphoplasmacytic inflammation also occurred in the cornea and often was accompanied by corneal edema and heterophilic infiltrates (Fig. 5). Nasal turbinate lesions consisted of submucosal infiltrates of lymphocytes and plasma cells as well as focal mucosal necrosis with heterophilic infiltrates. Tracheal mucosa and submucosa were thickened by epithelial hyperplasia and lymphoplasmacytic infiltrates (Fig. 6).

Microscopic lesions correlated well with gross lesions. Twenty-two birds without gross lesions were examined by histology.

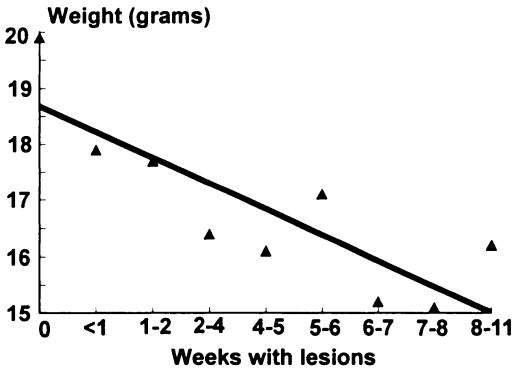

FIGURE 3. Mean weights for house finches (*Carpodacus mexicanus*) with conjunctival lesions caused by *Mycoplasma gallisepticum* during a 12 wk captive study.

TABLE 1. Results of histopathologic, culture, polymerase chain reaction (PCR), and serologic testing of house finches (*Carpodacus mexicanus*) naturally infected with *Mycoplasma gallisepticum*.

Number of weeks[a]	Microscopic lesions			Culture	PCR	Serology[b]		
	Conjunctiva	Turbinates	Trachea			SPA	HI	ELISA
Birds without gross lesions[c]								
0	6/22[d]	2/22	1/17	1/22	9/21	67/251	4/138	1/18
Birds with gross lesions								
<1	6/6	1/5	0/3	6/9	8/8	20/32	2/22	0/6
1–2	22/22	10/18	8/17	15/19	16/16	66/67	12/40	1/8
3–4	7/7	6/8	3/7	3/5	4/4	36/38	13/22	1/3
5–6	15/16	14/17	5/15	6/12	9/9	24/26	4/19	5/7
>6	15/16	11/16	3/13	7/10	6/6	10/12	1/8	6/10
Total	65/67	42/64	19/55	37/55	43/43	156/175	32/111	13[e]/34

[a] Number of weeks birds had gross conjunctival lesions.
[b] Serologic tests are serum plate agglutination (SPA), hemagglutination inhibition (HI), and enzyme-linked immunosorbent assay (ELISA).
[c] Includes uninfected birds, birds with early infections, and inapparent carriers.
[d] Number positive/number tested.
[e] Includes positive and equivocal results.

The majority of these birds were normal; however, mild to moderate microscopic lesions were present in six of 22 (27%) birds examined. Two of these birds had nasal turbinate or tracheal lesions that would not be apparent on gross inspection.

Mild to severe histologic lesions were present in the ocular tissues of birds with gross lesions, but sequential sampling of finches over time did not reveal an apparent progression of lesion severity. Mild to moderate nasal turbinate lesions were present in 42 of 64 (66%) birds with gross ocular lesions, whereas tracheal lesions were found in only 19 of 55 (35%) birds with gross lesions. Nasal turbinate and tracheal lesions developed within the first 2 wk of clinical disease; the prevalence and severity of lesions in the nasal turbinates and trachea apparently did not increase with gross lesion duration.

Mycoplasma gallisepticum was confirmed by culture and PCR in three of five finches with conjunctivitis that were tested at the beginning of the study. Culture and PCR results were compared between birds with and without gross conjunctival lesions sampled between 1 and 12 wk (Table 1). Of finches with gross lesions, *M. gallisepticum* was isolated from 37 of 55 (67%), and 43 of 43 (100%) were positive by PCR. In contrast, *M. gallisepticum* was isolated from only one of 22 (5%) birds without lesions, although nine of 21 (43%) tested by PCR were positive. Three of these birds remained alive and clinically normal for the entire study. *Mycoplasma gallisepticum* was cultured from birds that had lesions for 2 to 9 wk, and infection was detected by PCR for 10 wk after onset of lesions. The AP-PCR analysis comparing *M. gallisepticum* isolates from this study with field isolates associated with conjunctivitis in house finches showed that all finch isolates were identical yet differed from standard poultry strains (Fig. 7).

Positive cultures fermented six to 27 days after broth inoculation, but most grew between 10 to 16 days. Isolations were made from cultures of conjunctiva (71%), sinus (58%), trachea (68%), and lung and air sac (5%). Multiple isolations were made from 68% of culture-positive birds.

Antibodies to *M. gallisepticum* were detectable by SPA in 89% of birds with conjunctival lesions (Table 1). The development of SPA antibodies occurred concurrently with lesions in most finches, although several birds produced SPA

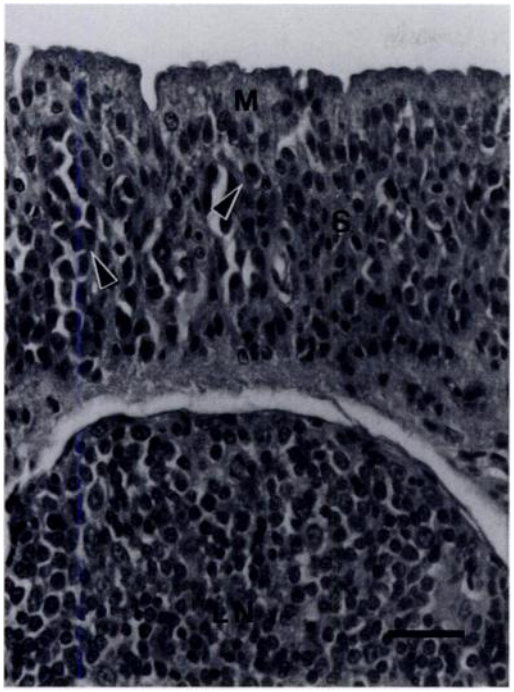

FIGURE 4. Conjunctiva of a house finch (*Carpodacus mexicanus*) infected with *Mycoplasma gallisepticum*. Plasma cells and lymphocytes (arrowheads) diffusely infiltrate the mucosa (m) and submucosa (s), and a lymphoid nodule (LN) is present deep within the submucosa. H&E. Bar = 60 μm.

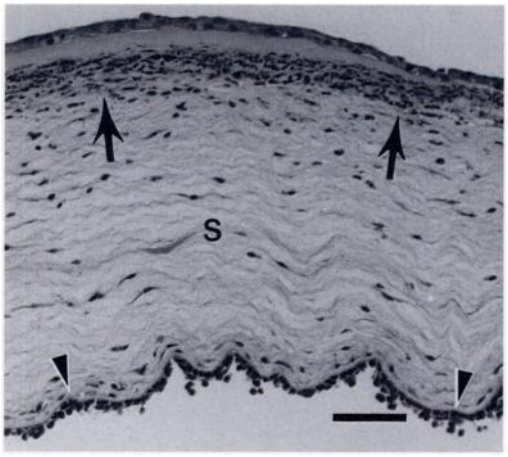

FIGURE 5. Cornea of a house finch (*Carpodacus mexicanus*) infected with *Mycoplasma gallisepticum*. The corneal stroma (s) is expanded by edema and contains a laminar infiltrate of heterophils (arrows) near the superficial surface. Low numbers of heterophils adhere to the endothelial aspect of Descemet's membrane (arrowheads). H&E. Bar = 60 μm.

reactivity 2 to 8 wk prior to the presence of lesions. Mean SPA scores increased from 0.95 in birds without lesions to 1.9 in birds with lesions of <1 wk duration and persisted at ≥3.1 in birds with lesions of >1 wk duration.

The HI and ELISA tests were less sensitive than the SPA test in detecting antibodies to *M. gallisepticum* in house finches (Table 1). There were numerous HI titers of 1:20, but positive titers of 1:40 or 1:80 were found only in 29% of birds with lesions and 3% of birds without lesions. Of 52 finches with and without lesions tested between 2 and 12 wk by ELISA, seven were positive, seven equivocal, and 38 negative. Six of seven ELISA-positive birds had conjunctival lesions for 4 wk or more, and those with equivocal results had lesions for 2 to 8 wk. All but one of these 14 birds also were SPA-positive, but there was no correlation between HI and ELISA results (data not shown).

Staphylococcus spp. were isolated from conjunctival swabs of seven of 10 birds at 6 wk, and 10 of 10 at 8 wk. Isolation attempts (n = 20) for *Haemophilus* spp. and

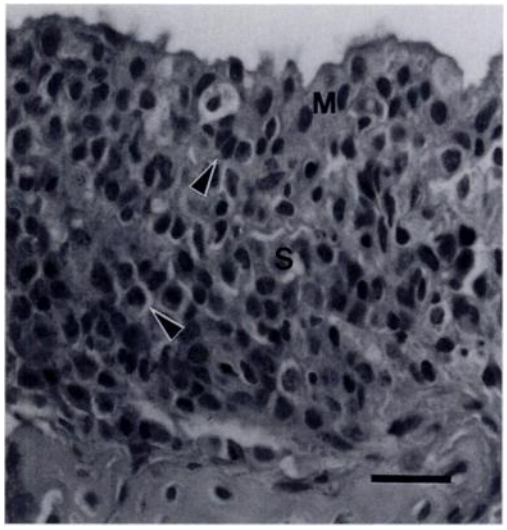

FIGURE 6. Trachea of a house finch (*Carpodacus mexicanus*) infected with *Mycoplasma gallisepticum*. The mucosa (m) and submucosa (s) are infiltrated by plasma cells, lymphocytes, and histiocytes (arrowheads). H&E. Bar = 20 μm.

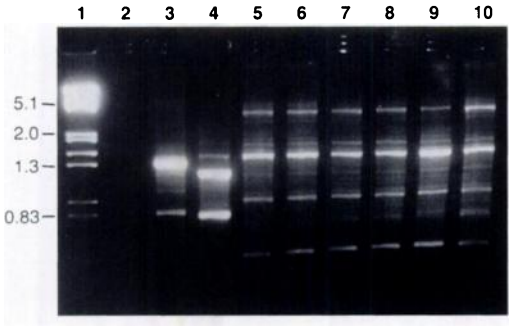

FIGURE 7. Electrophoretic analysis (2% agarose gel) of arbitrary primer polymerase chain reaction patterns of *Mycoplasma gallisepticum* isolates from house finches (*Carpodacus mexicanus*) with conjunctivitis. Lane 1 contains a molecular size marker made from bacteriophage lambda DNA digested with *Hin*dIII and *Eco*RI; numbers at left are molecular size markers in kb. Lane 2 is a negative control (reaction mixes without DNA). Poultry reference strains of *M. gallisepticum* are in lane 3 (R strain) and lane 4 (F strain). Lane 5 has an isolate cultured during the first week of the study, and lanes 6–8 have isolates from each of three pens at 12 wk. The remaining lanes have field isolates from Maryland (lane 9) and Georgia (lane 10).

Bordetella avium were unsuccessful. *Chlamydia* sp. was not detected in tissue imprints.

DISCUSSION

Most birds in this study developed clinical signs and lesions identical to those reported in field cases of mycoplasmal conjunctivitis in house finches (Ley et al., 1996; Luttrell et al., 1996). Infection with *M. gallisepticum* was characterized by unilateral or bilateral eyelid swelling, chronic conjunctivitis and rhinitis, weakness, weight loss, and often death. Clinical signs and lesions frequently were severe from the onset of disease, and there was no indication of remission in affected birds. Although three birds never developed clinical disease and *M. gallisepticum* was not cultured from them, results of PCR and serologic testing indicated that they had been exposed and probably were infected. It is possible that these birds would be asymptomatic carriers in the wild.

Infection with *M. gallisepticum* was detected in most birds by culture, PCR, or both; however, culture was less sensitive than PCR. Previous studies have reported difficulties in making primary isolations of *M. gallisepticum* from finches (Ley et al., 1996; Luttrell et al., 1996). Possible explanations for culture insensitivity include media failure, inhibitory substances in tissues, and the fastidious nature of the organism.

The SPA test detected the earliest antibody response in finches, and most birds continued to react on this test while infected. The HI response emerged 2 to 4 wk after birds became positive by SPA, which is similar to that observed in domestic poultry (Roberts, 1969) and wild turkeys (Rocke et al., 1985). There were numerous HI titers of 1:20 that may have tested higher with a homologous antigen. While more sensitive, the ELISA test generally detected antibody response only in birds that had been infected for longer than 4 wk. Because more serum (50 μl) is required for this ELISA procedure, our sample size was restricted. In addition, house finches may have a more variable immune response.

This finch-derived strain of *M. gallisepticum* spread quickly within the pens, and birds frequently became debilitated and died even though they were sheltered and fed ad libitum. Possible modes of transmission include direct contact between birds, exposure to contaminated feed and perches, and contamination from daily maintenance and weekly handling of birds. The transmission rate of *M. gallisepticum* within this captive population could not be determined since the number of infected birds at the beginning of the study was unknown. However, the extent of clinical disease and seroconversion observed over the 12 wk period suggests that *M. gallisepticum* is highly transmissible in this species. This also is supported by the rapid spread of the disease in free-flying finches since first detected in 1994 (Fischer et al., 1997).

Currently, the impact of mycoplasmal conjunctivitis on the eastern population of

house finches is unknown. Project FeederWatch, a backyard bird feeder survey sponsored by the Cornell Laboratory of Ornithology (Cornell University, Ithaca, New York, USA), has reported an increase in house finches with conjunctivitis throughout the eastern United States in 1994–95 (Dhondt, 1996). The highest number of observers reporting affected birds was in the Mid-Atlantic region (41%), where the epornitic originated; reported rates of lesions in regions to the north, south, and west of this nucleus were 7 to 23%. Laboratory confirmed diagnoses by the Southeastern Cooperative Wildlife Disease Study (Athens, Georgia, USA) and other diagnostic laboratories (Fischer et al., 1997) show that the disease has spread from the Mid-Atlantic states to include the entire range of the eastern house finch population. However, it may be several years before changes in population trends can be observed. The same strain of *M. gallisepticum* also has been confirmed in American goldfinches (*Carduelis tristis*) (Ley et al., 1997; M. P. Luttrell, unpublished data), so the potential for spread to other species, especially those feeding together in large groups, is apparent and warrants continued monitoring.

Due to the severity of clinical disease and the extended period of active *M. gallisepticum* infection, there is a need to assess the risk of transmission between infected house finches and other wild birds and domestic poultry. A better understanding of modes of transmission will aid in designing control programs for disease in wild birds and in preventing introductions of *M. gallisepticum* into commercial poultry flocks.

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