MYCOPLASMOSIS IN CAPTIVE CROWS AND ROBINS FROM MINNESOTA

Authors: Wellehan, James F. X., Calsamiglia, Maria, Ley, David H., Zens, Mark S., Amonsin, Alongkorn, et al.

Source: Journal of Wildlife Diseases, 37(3) : 547-555

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-37.3.547

Journal of Wildlife Diseases, 37(3), 2001, pp. 547–555

MYCOPLASMOSIS IN CAPTIVE CROWS AND ROBINS FROM MINNESOTA

James F. X. Wellehan,[1,2] Maria Calsamiglia,[1] David H. Ley,[4] Mark S. Zens,[2] Alongkorn Amonsin,[3] and Vivek Kapur[1,5]

[1] Department of Veterinary PathoBiology, [2] Wildlife Rehabilitation Center, and [3] Department of Clinical and Population Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota, 55108 USA
[4] Department of Farm Animal Health and Resources Management, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina 27606, USA
[5] Corresponding author (e-mail: vkapur@tc.umn.edu)

ABSTRACT: *Mycoplasma sturni* is a recently described organism previously associated with conjunctivitis in European starlings (*Sturnus vulgaris*), northern mockingbirds (*Mimus polyglottos*) and blue jays (*Cyanocitta cristata*). Herein we describe the isolation of *M. sturni* from an American crow (*Corvus brachyrhynchos*) presenting with conjunctivitis. A nested-PCR was designed for identification of *M. sturni* in clinical specimens and the sensitivity of the reaction was found to be 10 colony-changing units. The organism was found in asymptomatic American crows caged with a nestmate of the crow with conjunctivitis. *Mycoplasma sturni* also was found in asymptomatic American robins (*Turdus migratorius*) and in a European starling (*Sturnus vulgaris*) housed at the same facility as the crows. Heterogenity of *M. sturni* isolates from different host species was found by random amplified polymorphic DNA (RAPD) analyses. Heterogeneity also was found among *M. sturni* isolates recovered from American crows.

We suggest that *M. sturni* can successfully infect American crows and American robins with or without the presence of clinical disease. Furthermore, we demonstrate that nested-PCR is an effective method for the detection of *M. sturni* and that substantial genetic heterogeneity exists among natural isolates of this bacterial pathogen.

Key words: American robin, American crow, *Corvus brachyrhynchos*, *Mycoplasma sturni*, mycoplasmosis, nested polymerase chain reaction, *Turdus migratorius*.

INTRODUCTION

Mycoplasma sturni was first described in 1996 in a European starling (*Sturnus vulgaris*) presenting with bilateral conjunctivitis with mucocaseous discharge (Forsyth et al., 1996; Frasca et al., 1997). Since then, the organism has been found in mockingbirds (*Mimus polyglottos*) and a blue jay (*Cyanocitta cristata*) in the eastern USA (Ley et al., 1997). While all natural *M. sturni* infections described to date have been found in passeriform birds, the full host species range has yet to be determined.

The mycoplasmas rank among the most prevalent and costly infectious diseases of birds worldwide (Ley and Yoder, 1997). There are no clinical signs or lesions that are pathognomonic for any of 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. Culture of *Mycoplasma* spp. requires specialized media and is slow, requiring up to 4 wk (Jordan, 1996). A commonly used diagnostic test for the detection of *M. sturni* is indirect immunofluorescence (Ley et al., 1998), which is culture dependent and therefore takes at least several days to obtain results.

Polymerase chain reaction (PCR) is a culture-independent method of *M. sturni* identification. PCR technology is ideally suited for *Mycoplasma* spp. diagnosis because it is rapid and specific, does not require viable organisms, and may be automated.

We here describe the detection of *M. sturni* in an American crow (*Corvus brachyrhynchos*) with conjunctivitis, in apparently healthy American crows, and in apparently healthy American robins (*Turdus migratorius*). These isolates showed marked genetic heterogeneity by RAPD analyses. Further, we describe the development and application of a simple, sensitive, and specific nested-PCR for the detection of *M. sturni* in clinical specimens.

MATERIALS AND METHODS

Cultures

Choanal cleft and tracheal swabs (Calgiswab, Spectrum Laboratories, Houston, Texas, USA) were collected from birds admitted to the Wildlife Rehabilitation Center of Minnesota (WRC; St. Paul, Minnesota, USA). Swabs were pre-moistened in Frey's medium (Whitford et al., 1994) and cultured in 2 ml Frey's medium in a shaker incubator at 37 C for 24 to 48 hr. Cultures showing a pH change were filtered through a 0.45 μm filter (Gelman Sciences, Ann Arbor, Michigan, USA) into fresh Frey's media. All *M. sturni* isolates were collected from birds at the WRC and selected isolates were identified by 16S ribosomal DNA sequencing. For comparative analysis, we used *M. gallisepticum* collected from a house finch (*Carpodacus mexicanus*) and *M. gallopavonis* isolated from a wild turkey (*Meleagris gallopavo*) at the WRC. *Mycoplasma synoviae* and *M. iowae* isolates were graciously provided by S. Kleven (University of Georgia, Athens, Georgia, USA).

Birds

A nest containing two orphaned American crows (*Corvus brachyrhynchos*) was admitted at the WRC in July 1997. One crow (97–2147) presented with bilateral conjunctivitis and severe vestibular disease and was euthanized and a tracheal swab collected for *Mycoplasma* spp. culture. The asymptomatic nestmate (97–2148) was admitted to the avian nursery and shared a flight cage with ten other juvenile American crows. All of these crows were cultured for *Mycoplasma* spp. 2 wk later. Six American robins (*Turdus migratorius*) that had been in the nursery room and were transferred to a flight cage separated by netting from the crows also were cultured for *Mycoplasma* spp. A wild turkey (*Meleagris gallopavo*) that was kept in the same flight cage as the American robins also was cultured for *Mycoplasma* spp. The birds were held for ≤2 mo, and when no clinical disease developed they were released. A European starling without signs of conjunctivitis was found dead in the flight cage with the robins and was cultured for *Mycoplasma* spp.

Indirect immunofluorescence

Indirect immunofluorescence (IF) was performed using standard methods (Kleven and Yoder, 1989). The isolates were cultured on Frey's agar. Colonies were incubated with *M. sturni* antiserum prepared previously by S. Geary (University of Connecticut, Storrs, Connecticut, USA) to *M. sturni* type strain (UCMF American Type Culture Collection No. 51945) (Forsyth et al., 1996) for 30 min at 37 C, then washed three times with PBS (pH 7.2). Monoclonal anti-rabbit immunoglobulin-FITC conjugate (Sigma Chemical, St. Louis, Missouri, USA) was incubated for 30 min at 37 C, then washed three times with PBS (pH 7.2). The treated colonies were examined microscopically (Labophot, Nikon Inc., Melville, New York, USA) under low magnification using ultraviolet light and epiillumination.

Nested PCR

The 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. sturni* 16S rRNA (GenBank accession #U22013) (5′-CRAAYGGGTGAGTAACACGTA, Y = pyrimidine, R = purine) and primer 2, and 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, St. Paul, Minnesota, USA. The 20 μl reaction mixture contained swab material, PCR buffer I (Perkin-Elmer, Branchburg, New Jersey, USA), 5% glycerol, 0.4 μM for each primer, 200 μM for 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 30 sec, primer extension at 72 C for 30 sec, and a final extension step at 72 C for 7 min. For the second *M. sturni*-specific PCR amplification, 0.5 μl of product from the above reaction was then used in a second PCR using primer 1 and primer MST (5′ -CCGA-AGGCCGTCATCGCA). The 20 μl reaction mixture consisted of 2 μl of template, PCR buffer I (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 40 cycles of denaturation at 94 C for 60 sec, annealing at 56 C for 30 sec, primer extension at 72 C for 30 sec, and a final extension step at 72 C for 7 min. A 5 μl volume of PCR products 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 UV light using an Eagle Eye II gel documentation system (Stratagene, La Jolla, California).

The sensitivity of this nested PCR was determined by the following method: Frey's medium (Whitford et al., 1994) was inoculated with *M. sturni* strain 97–2147 (from the crow with conjunctivitis) and cultured in a shaker incubator at 37 C overnight. 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. Serial dilutions of the original culture were tested using the nested PCR method.

To confirm the results of the above procedure, a second method also was used. Since the genome size of *M. sturni* is approximately 870 kbp (Forsyth et al., 1996), the weight of one genome equivalent is approximately 8.81×10^{-10} μg. Genomic DNA was prepared from isolates using QIAamp tissue kit (Qiagen, Valencia, California, USA). The DNA was resuspended in TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 8.0) and quantified by spectrophotometry (Model Du-64, Beckman, Fullerton, California, USA). Serial dilutions of the DNA preparation with known genome equivalents were tested using the nested PCR method.

DNA sequencing

Sequencing reactions of 16S amplifications of three *M. sturni* isolates from crows were performed with the Ready Reaction Dye Terminator Kit (Perkin-Elmer), 4 pmol primer, and 100 ng PCR product, and analyzed on ABI 377 automated DNA sequencers at the AGAC.

RAPD analysis

Two ml of second-passage log phase broth cultures containing approximately 1×10^9 CFU were centrifuged at 16,000 *g* for 10 min, washed with PBS and resuspended in 25 μl PBS. The cells were lysed at 100 C for 10 min, chilled on ice for 5 min, and centrifuged at 16,000 *g* for 5 min to remove debris. Previously described RAPD methods (Fan et al., 1995; Geary et al., 1994) were used with modifications (Ley et al., 1997). The 100 μl reaction mixture contained 3 μl DNA extract with 300–500 ng DNA, 2.5 units Taq polymerase (Boehringer, Mannheim, Germany), buffer with 1.5 mM $MgCl_2$, 500 ng of each primer described by Fan et al. (1995) M16SPCR5 (5′ AGGCAGCAGTAGGGAAT), M13F (5′ GTAAAACGACGGC), and S1OLIGO (5′ CATAACTAACATAAGGGCAA) and 200 μM each of dATP, dCTP, dGTP, and dTTP. The mixtures were amplified for three cycles with a denaturation at 94 C for 15 sec, annealing at 28 C for 2 min, and primer extension at 74 C for 3 min. This was followed by 35 cycles with a denaturation at 94 C for 15 seconds, annealing at 45 C for 2 min, and primer extension at 74 C for 3 min. Amplified DNA was separated electrophoretically in 2% agarose gels, stained with ethidium bromide, illuminated with UV light and photographed using Polaroid 667 film. Photographs were digitized using a flat bed scanner (ScanJet 6300C, Hewlett Packard, Palo Alto, California), and the digital images labeled and edited using PhotoShop (Abode, San Jose, California). Images were inverted (black and white reversed) using PhotoShop to enhance visualization of faint bands.

Phylogenetic analysis

Mycoplasma sturni, *M. gallopavonis*, *M. synoviae*, *M. gallisepticum*, *M. iowae*, and *M. meleagridis* partial 16S rDNA sequences were compared with sequences submitted to GenBank National Institute of Health, Bethesda, Maryland, USA; accession numbers U22013, AF064062, X52083, L08896, U29676, and L24106, respectively). All sequences showed 100% homology with Genbank sequences. Genetic relationships based on comparison of 16S rDNA sequences from bases 102 to 506 as well as analogous GenBank sequences from *M. corogypsi* (L08054), *M. gallinaceum* (L24104), *M. gallinarum* (L24105), *M. imitans* (L24103), and *Ureaplasma gallorale* (U63937) were determined by MEGALIGN (DNASTAR, Madison, WI). A majority rule bootstrap tree was generated by using PAUP 4.0b2a (Swofford, 1998). The dendrogram was constructed using the nearest-neighbor interchange (NNI) option with branch-swapping and 1,000 bootstrap replications. *Mycoplasma gallisepticum*, *M. iowae*, *M. imitans*, and *U. gallorale* were used as an outgroup for outgroup rooting.

RESULTS

Mycoplasma sturni was cultured and identified by 16S rDNA sequencing and IF from the first crow admitted at the Wildlife Rehabilitation Center showing bilateral conjunctivitis and vestibular disease. Sequencing of the 16S rDNA from bases 102 to 506 showed 100% homology with the type strain *M. sturni* UCMF (Genbank accession number U22013). Although none of the eleven crows in the same cage, including the nestmate of the crow with conjunctivitis, developed con-

junctivitis, *M. sturni* was cultured and confirmed by PCR from eight (73%) of the cagemates. Six crow isolates were also tested by IF; all were positive. The other three of the eleven cagemates tested were found to be negative by both PCR and culture. The six American robins that had been in the nursery room and were transferred to a flight cage adjacent to the crows were also found to be positive for *M. sturni* by culture and PCR. One American Robin isolate was tested by IF and found to be positive. *M. sturni* was not detected by culture or PCR in the wild turkey that was housed in the same flight room as infected American robins. The birds were held for up to two months, and when no clinical disease developed, they were released. *Mycoplasma sturni* was identified by PCR and IF following culture from a tracheal swab of the European starling found dead in the flight room with the robins.

The nested PCR produced a specific 327 base pair band when purified *M. sturni* DNA was used as a template, and also from all clinical specimens which consisted of one European starling, eight American crows, and six American robins. The nested-PCR did not amplify samples of *M. synoviae*, *M. gallisepticum*, *M. iowae*, or *M. gallopavonis* (Fig. 1A), and had a detection limit of approximately 10 organisms using both procedures described in the materials and methods section (Fig. 1B).

In the RAPD analysis (Fig. 2) the American crow isolates in lanes 4 and 5 have the same banding pattern. The American crow isolates in lanes 6 through 9 have a similar but distinct banding pattern. Lane 8 is from the nestling crow with conjunctivitis, and lane 9 is from its apparently healthy nestmate. The European starling isolate in lane 10 has a banding pattern distinct from the American crow isolates but very similar to the control mockingbird isolate from Florida. The American robin isolate in lane 11 has a banding pattern distinct from the other isolates. DNA fingerprints of isolates from different hosts are distinct. Host intraspecies differences are seen in American crow isolates.

Bootstrap analysis grouped *M. sturni* with *M. gallopavonis*, *M. gallinaceum*, *M. coragypsi*, and *M. synoviae* (Fig. 3). Although not distinguished by bootstrap analysis, *M. sturni* is closest to *M. gallopavonis* from bases 102 to 506, with 92% homology. *Mycoplasma gallopavonis* is an organism commonly found in wild turkeys and is generally considered nonpathogenic (Hoffman et al., 1997).

DISCUSSION

To our knowledge, this is the first report of *M. sturni* identified in samples from American crows and American robins. All reported natural *M. sturni* infections to date have been described in other passeriform birds. The full host species range has yet to be determined.

In this paper, we describe a nested PCR assay to detect *M. sturni* from clinical samples. The 16S rRNA gene was chosen as a PCR template because of its high degree of conservation. This assay has several advantages over other assays for use in wildlife. PCR 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 post-mortem in less than optimal condition and many *Mycoplasma* spp. do not remain viable.

Serological tests also are commonly used for diagnosis of *Mycoplasma* spp. PCR has been shown to be more sensitive than serology in some investigations (Buck et al., 1995; Blanchard et al., 1996; Kempf et al., 1997), and it is generally accepted that it is at least as sensitive. Nested PCR is more sensitive than simple PCR (Miserez et al., 1997). Another difficulty with serology is that any mycoplasmal protein recognized by the immune system is under selective pressure to change (Le Grand et al., 1996; Droesse et al., 1995). Additionally, ELISA is considered to be the pre-

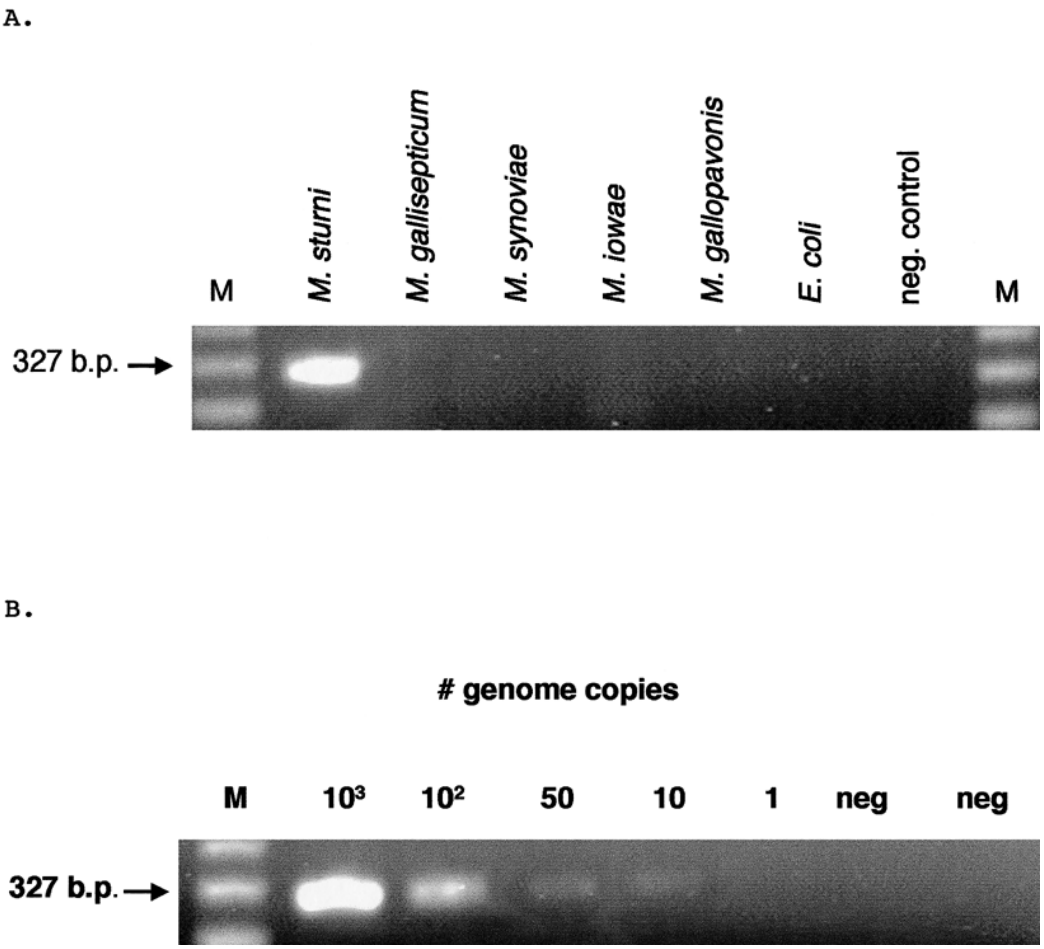

FIGURE 1. A Specificity of nested PCR. Products of *M. sturni* nested PCR reactions for different avian *Mycoplasma* spp. as templates are presented in lanes 2 through 8. M, 123 bp marker. B. Sensitivity of nested PCR. Products of nested PCR reactions of different dilutions of *M. sturni* are presented in lanes 2 through 8. M, 123 bp marker.

mier serological test for detection of *Mycoplasma* spp. (Ewing et al., 1996). Most ELISA assays are indirect, requiring a secondary enzyme-linked antibody that binds to the F_c portion of the target antibody. While these secondary antibodies are typically available for domestic species, this poses a problem for use in wildlife.

We suggest possible horizontal transmission of *M. sturni* among American crows. A bird from an infected nest was housed in a room with other birds that later were shown to be infected. Potential vectors for horizontal transmission include direct contact, handlers, and fomites. However, horizontal transmission has not been conclusively demonstrated. The infection status of all birds in the flight rooms upon admission was not known, and there may be a very high prevalence of the organism in wild passerines. *Mycoplasma sturni* isolates distinguishable by RAPD analysis were found in American robins and European starlings that were cultured because of their proximity to the American crows. The closely related *M. gallopavonis* has been shown to have a very high prevalence in wild turkeys (Hoffman et al., 1997). Monitoring and biosecurity practices are advisable to prevent the spread of

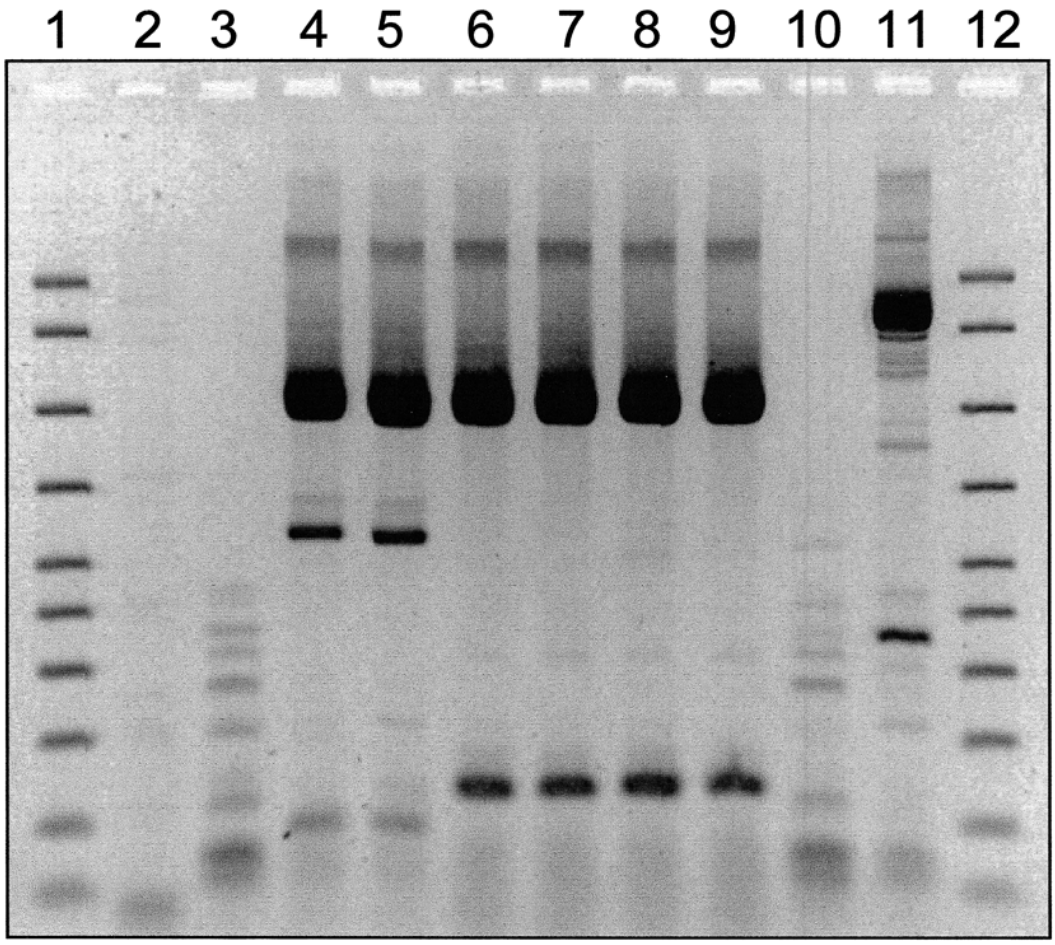

FIGURE 2. RAPD DNA fingerprinting. Amplified DNA was separated electrophoretically in 2% agarose gels, stained with ethidium bromide, illuminated with UV light and photographed. The photograph was digitized using a flat bed scanner and inverted (black and white reversed to enhance visualization of faint bands). Lanes 4–11 represent *M. sturni* isolates made at the WRC. Lane 1 is the DNA Ladder (AmpliSize Molecular Ruler, Bio-Rad Laboratories, Hercules, California, USA) at 2,000, 1,500, 1,000, 700, 500, 400, 300, 200, 100, 50 bp.; Lane 2 is the Negative control; Lane 3 is the *M. sturni* isolate 17194–5 from a Florida mockingbird (Ley et al., 1998); Lane 4 is the American crow 97–1806 *M. sturni;* Lane 5 is the American crow 97–1944 *M. sturni;* Lane 6 is the American crow 97–1953 *M. sturni;* Lane 7 is the American crow 97-2013 *M. sturni*; Lane 8 is the American crow 97-2147 *M. sturni*; Lane 9 is an American crow 97-2148 *M. sturni*; Lane 10 is the European starling 97-2642 *M. sturni*; Lane 11 is the American robin 97-3345 *M. sturni*; and Lane 12 is the AmpliSize DNA Ladder

M. sturni and other organisms within rehabilitation facilities.

Mycoplasma sturni was not detected in a wild turkey (*Meleagris gallopavo*) that was kept in the same flight room as infected American robins. However *M. gallopavonis* was cultured from this turkey. *Mycoplasma gallopavonis* is the most closely related organism to *M. sturni* according to 16S-sequence homology, and there may be some antigenic similarity. Four possible explanations for not detecting *M. sturni* in the turkey are (1) failure to detect the organism present in this bird, (2) that the turkey was not infected by chance, (3) that *M. gallopavonis* and *M. sturni* are antigenically similar enough to provide cross-protection, or (4) that wild

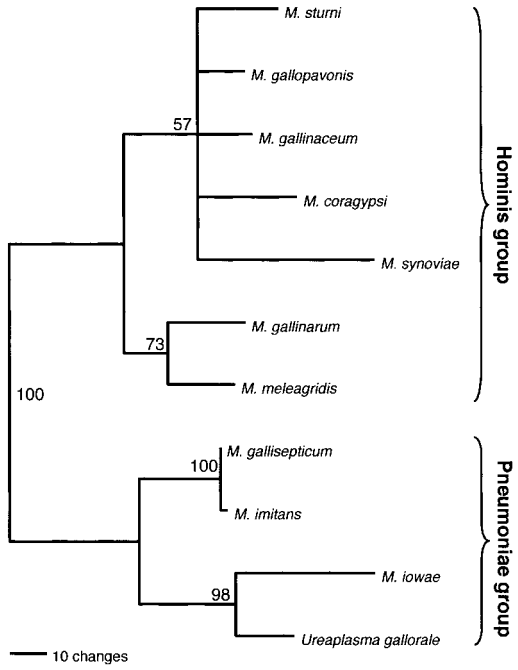

FIGURE 3. Phylogram of *Mycoplasma* spp. using comparison of 16s rDNA from bases 102 to 506. The majority rule bootstrap tree was generated by using PAUP 4.0b2a (Swofford, 1998). The dendrogram was constructed using the nearest-neighbor interchange (NNI) option with branch-swapping and 1,000 bootstrap replications. *Mycoplasma gallisepticum*, *M. iowae*, *M. imitans*, and *U. gallorale* were used as an outgroup for outgroup rooting. Bootstrap values are given.

turkeys are an unsuitable host for *M. sturni*.

Weisburg et al. (1989) have proposed a classification scheme for the mycoplasmas based on 16S rRNA gene sequences. The results show surprising diversity. According to this classification system, five groups were recognized. *M. synoviae* falls into the hominis group, and *M. sturni* should be placed in this group. *M. gallisepticum* falls into pneumoniae group and are evolutionarily quite distinct. The clinical implications of this are not known, but there may be pharmacological differences in drug response.

The provenance of *M. sturni* is not known. While all previous reports of isolation have been from the eastern U.S. (Forsyth et al., 1996; Ley et al., 1998), the results of these investigations show that *M. sturni* is found in birds in the upper midwestern United States. The other species associated with conjunctivitis in wild songbirds, *M. gallisepticum*, has a high prevalence of disease in infected birds (Luttrell et al., 1998). It is possible that disease prevalence may not be as high for *M. sturni*. A low prevalence of disease and the difficulty of culture would make it easy for *M. sturni* to evade identification. In fact, without the investigations due to the recent house finch *M. gallisepticum* epidemic, *M. sturni* might still be unidentified. Isolates of *M. gallisepticum* from the recent outbreak in wild passerines show no detectable differences using molecular epidemiology techniques, suggesting recent origin and rapid spread from the eastern United States (Fischer et al., 1997; Ley et al., 1997). *M. sturni* has already shown greater diversity by RAPD, suggesting a less recent origin.

The virulence of this organism and its association with disease remains to be determined. Other reports of *M. sturni* isolations have all been associated with conjunctivitis. To our knowledge, this is the first report of *M. sturni* isolation from apparently healthy birds. Only one of the nine (11%) infected American crows and none of the six infected American robins showed obvious signs of illness. Although *M. gallisepticum* was not detected by nested PCR (not shown) in the culture of the American crow with conjunctivitis, *M. gallisepticum* coinfection cannot be ruled out; *M. sturni* grows much more rapidly in laboratory culture than *M. gallisepticum* and potential overgrowth of *M. sturni* would make detection of *M. gallisepticum* difficult. Infection of another corvid (a blue jay) with house finch strains of *M. gallisepticum* has been reported (Ley et al., 1997).

Herein, we describe the detection of *M. sturni* in two new host species using a simple, sensitive, and specific nested-PCR. This test may aid in future studies of the host range, geographical range, and prevalence of *M. sturni.* This also is the first report of natural *M. sturni* infections in apparently healthy birds; further studies of pathogenicity are merited. We demonstrated that these isolates showed marked genetic heterogeneity by RAPD analyses. Further molecular epidemiological studies are required to determine the genetic diversity of *M. sturni.*

ACKNOWLEDGEMENTS

The authors thank L. Li, A. Bach, G. Rajashekara, B. May, and the volunteers of the Wildlife Rehabilitation Clinic for their assistance. We also thank S. Kleven and J. Newman for providing poultry mycoplasma isolates and S. Geary for providing antisera.

LITERATURE CITED

ABELE-HORN, M., C. WOLFF, P. DRESSEL, A. ZIMMERMAN, W. VAHLENSIECK, F. PFAFF, AND G. RUCKDESCHEL. 1996. Polymerase chain reaction versus culture for detection of *Ureaplasma urealyticum* and *Mycoplasma hominis* in the urogenital tract of adults and the respiratory tract of newborns. European Journal of Clinical Microbiology and Infectious Diseases 15: 595–598.

BLANCHARD B., M. KOBISCH, J. M. BOVE, AND C. SAILLARD. 1996. Polymerase chain reaction for *Mycoplasma hyopneumoniae* detection in tracheobronchiolar washings from pigs. Molecular and Cellular Probes 10: 15–22.

BUCK, G. E., AND N. S. EID. 1995. Diagnosis of *Mycoplasma pneumoniae* pneumonia in pediatric patients by polymerase chain reaction (PCR). Pediatric Pulmonology 20: 297–300.

DROESSE M., G. TANGEN, I. GUMMELT, H. KIRCHHOFF, L. R. WASHBURN, AND R. ROSENGARTEN. 1995. Major membrane proteins and lipoproteins as highly variable immunogenic surface components and strain-specific antigenic markers of *Mycoplasma arthritidis.* Microbiology 141: 3207–3219.

EWING, M. L., L. H. LAUERMAN, S. H. KLEVEN, AND M. B. BROWN. 1996. Evaluation of diagnostic procedures to detect *Mycoplasma synoviae* in commercial multiplier-breeder farms and commercial hatcheries in Florida. Avian Diseases 40: 798–806.

FAN, H. H., S. H. KLEVEN, AND M. W. JACKWOOD. 1995. Application of polymerase chain reaction with arbitrary primers to strain identification of *Mycoplasma gallisepticum.* Avian Diseases 39: 729–735.

FISCHER, J. R., D. E. STALLKNECHT, M. P. LUTRELL, A. A. DHONT, 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.

FORSYTH, M. H., J. G. TULLY, T. S. GORTON, L. HINKLEY, S. FRASCA JR., H. J. VAN KRUININGEN, AND S. J. GEARY. 1996. *Mycoplasma sturni* sp. nov., from the Conjunctiva of a European starling (Sturnus vulgaris). International Journal of Systematic Bacteriology 46: 716–719.

FRASCA, S., JR., L. HINCKLEY, M. H. FORSYTH, T. S. GORTON, S. J. GEARY, AND H. J. VAN KRUININGEN. 1997. Mycoplasmal Conjunctivitis in a European Starling. Journal of Wildlife Diseases 33: 336–339.

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 & Cellular Probes 8: 311–316.

HOFFMAN, R. W., M. P. LUTTRELL, W. R. DAVIDSON, AND D. H. LEY. 1997. Mycoplasmas in wild turkeys living in association with domestic fowl. Journal of Wildlife Diseases 33: 526–535.

JORDAN, F. T. W. 1996. Avian mycoplasmosis. *In* Poultry diseases, F. T. W. Jordan and M. Pattison (eds.). W. B. Saunders Company Ltd., Philadelphia, Pennsylvania, pp. 81–93.

KEMPF, I., F. GESBERT, AND M. GUITTET. 1997. Experimental infection of chickens with an atypical *Mycoplasma gallisepticum* strain—comparison of diagnostic methods. Research in Veterinary Science 63: 211–213.

KLEVEN, S. H., AND H. W. YODER JR. 1989. Mycoplasmosis. *In* A laboratory manual for the isolation and identification of avian pathogens, H. G. Purchase, L. H. Arp, C. H. Domermuth, and J. E. Pearson (eds.). American Association of Avian Pathologists, Kennett Square, Pennsylvania, pp. 57–62.

LE GRAND, D., M. SOLSONA, R. ROSENGARTEN, AND F. POUMARAT. 1996. Adaptive surface antigen variation in *Mycoplasma bovis* to the host immune response. FEMS Microbiology Letters 144: 264–275.

LEY, D. H., J. E. BERKHOFF, AND S. LEVISOHN. 1997. Molecular Epidemiologic Investigations of *Mycoplasma gallisepticum* Conjunctivitis in Songbirds by Random Amplified Polymorphic DNA Analyses. Emerging Infectious Diseases 3: 375–380.

———, S. J. GEARY, J. E. BERKHOFF, J. M. MCLAREN, AND S. LEVISOHN. 1998. *Mycoplasma sturni* from blue jays and northern mockingbirds

with conjunctivitis in Florida. Journal of Wildlife Diseases 34: 403–406.

———, AND H. W. YODER. 1997. *Mycoplasma gallisepticum* infection. *In* Disease of poultry, B. W. Calnek (ed.). Iowa State University Press, Ames, Iowa, pp. 194–207.

LUTTRELL, M. P., 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.

MISEREZ, R., T. PILLOUD, X. CHENG, J. NICOLET, C. GRIOT, AND J. FREY. 1997. Development of a sensitive nested PCR method for the specific detection of *Mycoplasma mycoides* subsp. mycoides SC. Molecular and Cellular Probes 11: 103–111.

SANCHEZ, S., K. TYLER, N. ROZENGURT, AND J. LIDA. 1994. Comparison of a PCR-based diagnostic assay for *Mycoplasma pulmonis* with traditional detection techniques. Laboratory Animals 28: 249–256.

SASCHE, K., H. PFUTZNER, H. HOTZEL, B. DEMUTH, M. HELLER, AND E. BERTHOLD. 1993. Comparison of various diagnostic methods for the detection of *Mycoplasma bovis.* Revue Scientifique et Technique 12: 571–580.

SWOFFORD, D. L. 1998. PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts, (Computer Program, not paginated).

TOLA, S., A. ANGIOI, A. M. ROCCHIGIANI, G. IDINI, D. MANUNTA, G. GALLERI, AND G. LEORI. 1997. Detection of *Mycoplasma agalactiae* in sheep milk samples by polymerase chain reaction. Veterinary Microbiology 54: 17–22.

WEISBURG, W. G., J. G. TULLY, D. L. ROSE, J. P. PETZEL, H. OYAIZU, D. YANG, L. MANDELCO, J. SECHREST, T. G. LAWRENCE, J. VAN ETTEN, J. MANILOFF, AND C. R. WOESE. 1989. A phylogenetic analysis of the Mycoplasmas: Basis for their classification. Journal of Bacteriology 171: 6455–6467.

WHITFORD, H.W., R. F. ROSENBUSCH, AND L. H. LAUERMAN. (Editors). 1994. Mycoplasmosis in animals: Laboratory diagnosis; compiled by the Mycoplasmosis Committee of the American Association of Veterinary Laboratory Diagnosticians. Iowa State University Press, Ames, Iowa, 173 pp.

Received for publication 10 August 2000.