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Association of *Mycoplasma corogypsi* and Polyarthritis in a Black Vulture (*Coragyps atratus*) in Virginia

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ABSTRACT: On 10 October 2007, a Black Vulture (*Coragyps atratus*) was presented to the Wildlife Center of Virginia, Waynesboro, Virginia, USA, because of an inability to fly. Examination revealed multiple swollen, fluctuant joints. The bird suffered from lead toxicosis and had a prominent leukocytosis. Histopathologic evaluation revealed an acute fibrinoheterophilic polyarthritis, and results of routine aerobic and anaerobic culture of joint fluid were negative, although *Mycoplasma* sp. sequence-specific polymerase chain reaction was positive. Amplification of a portion of the 16S rRNA and subsequent phylogenetic analysis of the amplicon identified *Mycoplasma corogypsi*. This is the first report of polyarthritis being diagnosed in association with a *Mycoplasma* sp. in a vulture species. However, fulfilling Koch's postulates through experimental infections is required to draw conclusions concerning an etiologic diagnosis.

Key words: Black Vulture, *Coragyps atratus*, lead toxicosis, *Mycoplasma corogypsi*, polyarthritis, polymerase chain reaction, Virginia.

Infectious polyarthritis of free-ranging wild birds has been infrequently reported in the literature, although it is well-documented in the poultry industry. *Mycoplasma synoviae* is a well-known pathogen of domestic poultry, causing upper respiratory tract infections and infectious synovitis, often affecting multiple joints and tendon sheaths (Kleven, 2003). *Mycoplasma* species have been identified in association with polyarthritis in multiple domestic avian and mammalian hosts; however, not in wild birds (Walker, 2004).

Little is known regarding the occurrence, pathogenicity, and distribution of mycoplasmas in free-living raptors, although many *Mycoplasma* species have been isolated. Some of these isolations were from asymptomatic birds, whereas a few have been associated with clinical signs and the majority of these disease-associated cases manifest clinically as respiratory disease. Among vultures, isolation of mycoplasmas have been most frequently reported in Old World species. *Mycoplasma gypis* was isolated from a Eurasian Griffon (*Gyps fulvus*) with respiratory disease (Poveda et al., 1994). *Mycoplasma gallinarum* has been isolated from the upper respiratory tract of this species (Loria et al., 2008), and several other unidentified mycoplasmas have been cultured from Eurasian Griffons in Europe (Poveda et al., 1990a, b; Loria et al., 2008). *Mycoplasma vulturii* was isolated from pooled lung and spleen samples from an Oriental White-backed Vulture (*Gyps bengalensis*) that died from apparent diclofenac intoxication (Oaks et al., 2004). A Cinereous Vulture (*Aegypius monachus*) with histopathologic inflammatory change in the upper respiratory tract had *M. gallinarum* isolated from a tracheal swab (Poveda et al., 1990b).

Reports of mycoplasmas from New World vultures has been limited to *Mycoplasma corogypsi*, which was first isolated and characterized from a footpad abscess found on a free-living Black Vulture (*Coragyps atratus*) in Alabama, USA (Panangala et al., 1993). *Mycoplasma corogypsi* has also been cultured from

the trachea of asymptomatic captive Peregrine Falcons (*Falco peregrinus*), Saker Falcons (*Falco cherrug*), and gyr-saker hybrids in the Middle East (Lierz et al., 2002). Here, we report a polyarthritis associated with *M. corogypsi* in a free-living Black Vulture in Virginia.

On 10 October 2007, a Black Vulture was captured in Waynesboro, Virginia (38°4′N, 78°53′W) after being observed unable to fly and was transported to the Wildlife Center of Virginia. The bird weighed 1.45 kg; was severely lame, emaciated, and lethargic; had dull mentation; and suffered from severe ectoparasitism. Warm, fluctuant swellings were observed at the left carpal joint, left hock joint, and right tarsal joint.

The bird was anesthetized for diagnostic evaluation. Whole blood was collected for a complete blood count (CBC), serum biochemistry, and lead analysis. The serum biochemistry was unremarkable, whereas the CBC revealed a severe anemia (packed cell volume of 19) and a moderate leukocytosis (30,540 cells/μl), characterized by a heterophilia and monocytosis. Whole blood lead levels were 3.1 ppm, indicating lead toxicosis (Minnesota Veterinary Diagnostic Laboratory, St. Paul, Minnesota, USA). Aseptic arthrocentesis was performed on the left hock joint. Approximately 2 ml of cream-colored viscous material was removed from the joint and submitted for aerobic and anaerobic bacterial culture, both of which exhibited no growth. A *Mycoplasma* sp. was detected in the joint exudate by sequence specific polymerase chain reaction (PCR) using methods described previously (Feldman et al., 2006). Whole-body radiographs showed severe osteolytic change in the affected joints, and the bird was subsequently euthanized and necropsied the same day.

Gross examination at necropsy revealed the three abscessed joints distended with viscous, cream-colored liquid and occasional solid, pale yellow, friable masses (Fig. 1). The associated fibrous joint capsules and synovial membranes were thickened, and associated tendon sheaths were also involved. A solitary 0.5-cm granuloma was discovered in the pulmonary parenchyma, and moderate splenomegaly was present. Formalin-fixed samples of spleen, kidney, testis, adrenal gland, brain, heart, skeletal muscle, lung, ventriculus, proventriculus, small intestine, large intestine, and hock joint were examined histopathologically. Sections of the fixed tissues were embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin. In addition, selected tissues were stained with acid-fast bacillus (AFB) stain, Gomori methylamine silver (GMS) stain, Gram stain, and Congo red stain. A paraffin-embedded slide of the affected carpal joint was stained with Hoechst 33258 and Giemsa stains.

Abundant heterophilic debris and fibrin were adherent to the synovial membrane of the hock joint. The subsynovial connective tissue was multifocally infiltrated by numerous viable heterophils with fewer macrophages (Fig. 2). These findings were consistent with marked, acute fibrinoheterophilic arthritis. A focally extensive heterophilic bronchitis and prominent lymphoid hyperplasia of the bronchus-associated lymphoid tissue was noted in the lungs. The remainder of the lung tissue was within normal limits. A moderate splenic amyloidosis was present, consistent with chronic inflammation. The remaining submitted tissues, including the kidneys, revealed no significant histopathologic findings. Infectious agents were not detected in the spleen or any of the inflammatory lesions when evaluated with AFB stain, GMS stain, or Gram stain. Hoechst stain of the left carpal joint revealed multiple, small, distinct foci of nucleic acid present along the synovium (Fig. 3).

Phylogenetic analyses of partial 16S rRNA gene sequence (446 nucleotides) were performed. A basic alignment sequence tool (BLAST) search of the National Institutes of Health GenBank identified similarities to several 16S rRNA accessions
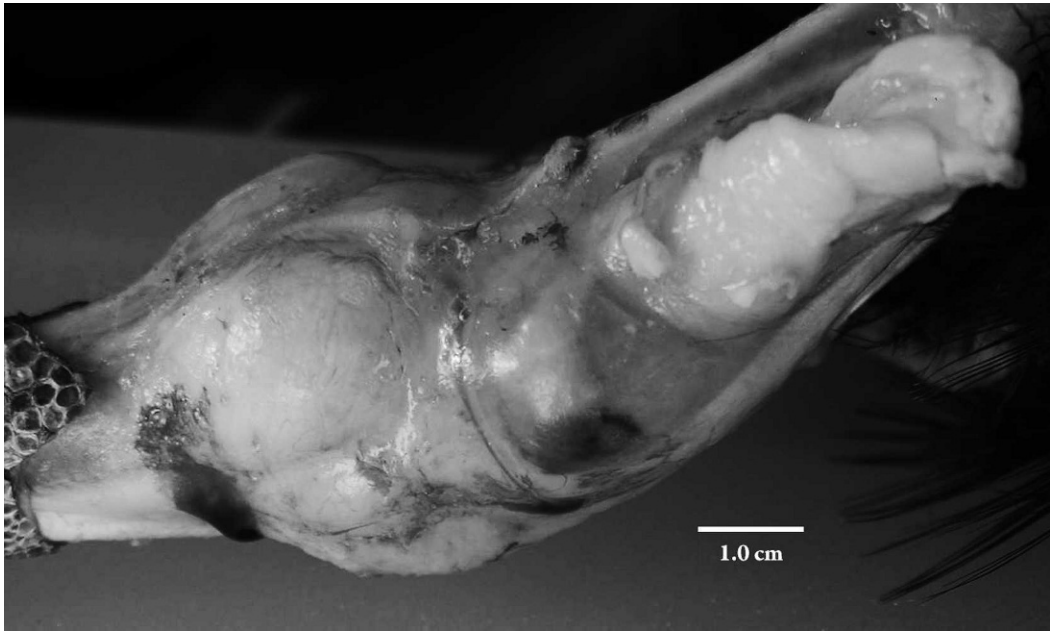
FIGURE 1.   Lateral view of the left hock joint of a Black Vulture (*Coragyps atratus*) with polyarthritis during postmortem examination. Overlying skin has been reflected to reveal severe distension of the joint.

from *Mycoplasma* species. GenBank accessions used in the analyses were as follows: *Mycoplasma alderi* (U67943), *M. agalactiae* 1 (AF332749), *M. agalactiae* 2 (AF010477), *M. alligatoris* (U56733), *M. anatis* (AF412970), *M. bovirhinis* (U44766), *M. bovis* strain 120/81 (AF332756), *M. buteonis* (AF412971), *M. canis* 1 (AF340023), *M. canis* 2 (AF412972), *M. capricolum* (AF170102), *M. citrelli* (AF412973), *M. columborale* (AF412975), *M. hyosynoviae* (U26730), *M. arthriditis* (M24580), *M. corogypsi* (L08054), *M. cricetuli* (EF036469), *M. crocodyli* (U63137), *M. gatae* (U15796), *M. cynos* 1 (AF412978), *M. cynos* 2 (AF538682), *M. edwardii* (U73903), *M. falconis* (AF125591), *M. felis* ATCC 23391 (U09787), *M. gallinaceum* (L24104), *M. gallinarum* (L24105), *M. gallisepticum* (M22441), *M. gallopavonis* 1 (AF064062), *M. gallopavonis* 2 (AF412980), *M. gateae* (U15796), *M. glycophilium* (AF412981), *M. gypis* (AF125589), *M. leonicaptivi* (U16759), *M. meleagridis* (L24106), *M. mustelae* (AF412986), *M. opalescens* (AF538961), *M. oxoniensis* (AF412987), *M. pullorum* (U58504), *M.* species 10T4 (AM182896), *M.* species 11CL2 (AM182893), *M.* species 39CL (AM182895), *M.* species 96430 (AF150495), *M.* species MS02 (DQ-223546), *M.* species (AJ000494), *M. spermatophilum* 1 (AF221119), *M. spermatophilum* 2 (AF013996), *M. sphenisci* (AY756171), *M. sturni* (U22013), *M. synoviae* (X52083), *M. vulturii* (AY191226), and *Escherichia coli* (AY776275). DNA sequences were aligned using ClustalX software (Thompson et al., 1997) and exported as both NEXUS and PHYLIP interleaved files. The GenBank sequences were truncated so that the 5′ and 3′ ends were coincident with the amplicon studied. Phylogenetic analysis was performed by two methods: Bayesian inference (Hulsenbeck and Ronquist, 2001) and maximum-likelihood (Felsenstein, 1989; Fig. 4).

For Bayesian analysis, the following parameters were used: general time reversal model with gamma-distributed rate variation, 100,000 generations of split sampling every 100th generation with a
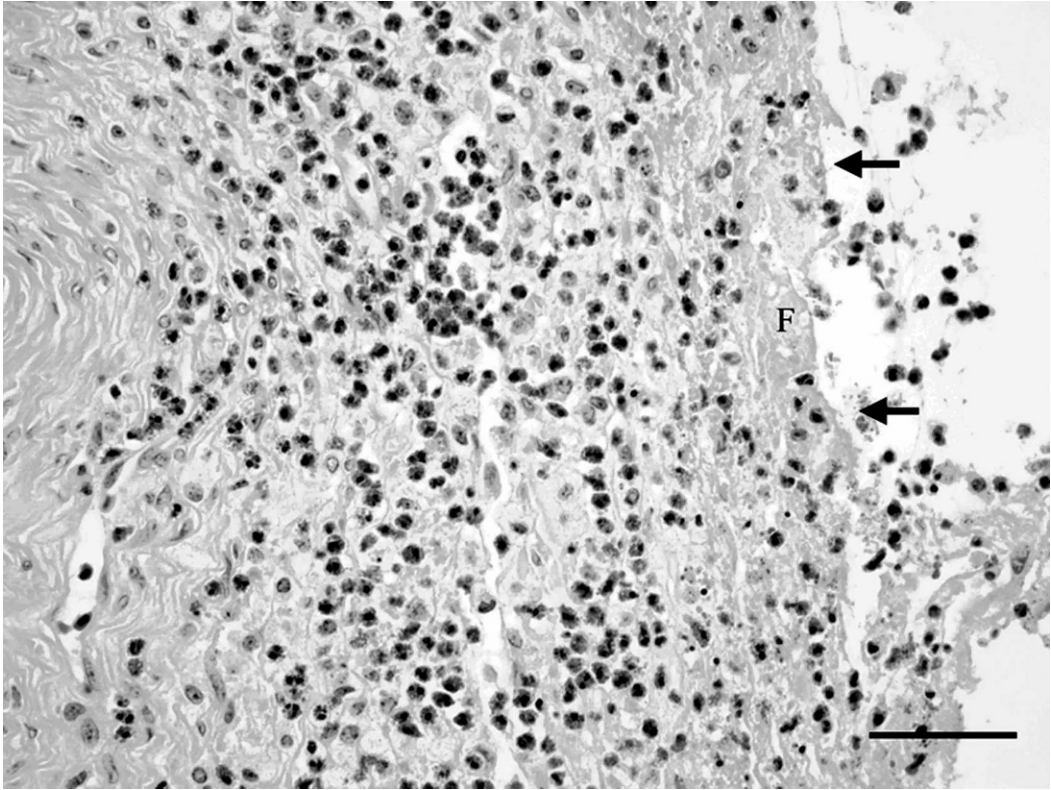
FIGURE 2.   Photomicrograph of the left hock joint showing abundant heterophilic debris and fibrin (F) adherent to the synovial membrane and multifocal infiltration of the subsynovial connective tissue by numerous viable heterophils with fewer macrophages. The arrows indicate the luminal surface of the inflamed synovial membrane. Bar=200 µm (hematoxylin and eosin).
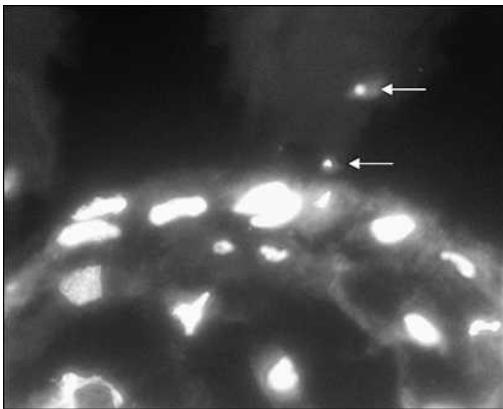

FIGURE 3.   Photomicrograph of the left carpal joint using Hoechst 33258 stain revealed multiple, small, distinct foci of nucleic acid present along the synovium (arrows). 400× total magnification.

convergence diagnostic rejecting the first 25% of values. The analysis was terminated when stationarity was obtained by observing the log probability plot vs. generation. For maximum-likelihood analysis using the PHYLIP inference software, 100 data sets were generated using SEQBOOT, and bootstrap analysis was performed jumbling input sequence order three times, using global rearrangement of sequences and the analysis set to slow and exact. The consensus phylogram was determined using CONSENSE. Phylograms generated by both methods were not time constrained, were rooted using *E. coli* as the outgroup and then displayed using TREEVIEW (Page, 1996). Regarding the maximum-likelihood analysis, results were similar to those delineated by Bayesian inference, but there were fewer
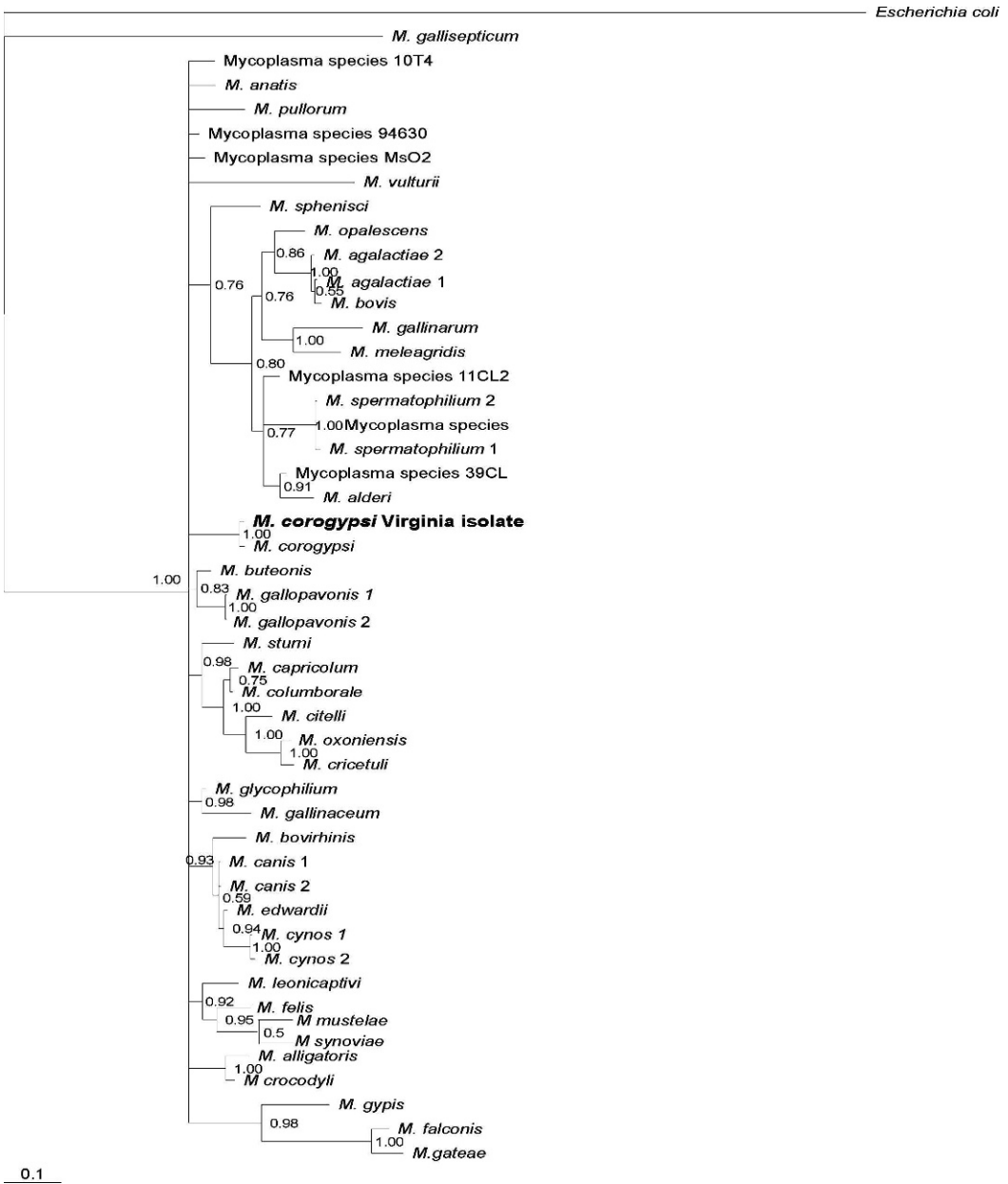
FIGURE 4.   Phylogram comparing known pathogenic *Mycoplasma* spp. with the 16S rRNA sequence of the *Mycoplasma corogypsi* isolated from the synovial fluid of a Black Vulture (*Coragyps atratus*) with polyarthritis using Bayesian inference.

terminal branches that were statistically significant. Bayesian inference gave more detailed information about relatedness amongst species, as shown in Figure 4. Bayesian inference retained branchpoints (nodes) that had at least 50% posterior probability.

Conclusive evidence that *M. corogypsi* is a pathogen of Black Vultures causing polyarthritis would require controlled laboratory studies; however, the results of our diagnostic evaluation are suggestive of an association between the presence of this bacterium in the synovial compartment and

the development of septic arthritis. No growth was observed on aerobic or anaerobic culture of synovial fluid from an affected joint; moreover, no microorganisms were seen on histopathologic examination of this joint using AFB stain, GMS-stain, and Gram stain. DNA was isolated from a synovial fluid aspirate when subjected to a generic mycoplasma PCR amplification and yielded a 446-base pair amplicon, indicating the presence of *Mycoplasma* DNA. The degenerate primer sequences were derived from alignments of several mycoplasmas, ureaplasmas, and acholeplasmas and are as follows: forward 5′-GYR-AAYGGGTGAGTAACACG and reverse 5′-CCTATGTATTACCGCGRCTG, where Y=C or T and R=A or G. The PCR reaction conditions were 95 C for 14 min 30 sec hotstart; 35 iterations of 95 C for 30 sec, 58 C for 30 sec, and 72 C for 1 min; and 72 C for 5 min final extension. The 25-μl reaction contained 2 mM $MgCl_2$, Hot-Star *Taq* polymerase (QIAGEN, Valencia, California, USA), 0.5 μM each degenerate primer, and 100 ng of specimen DNA. The sequence was determined for the amplicon (Fig. 5) and was submitted for a basic sequence alignment tool (BLAST) search of the GenBank database and returned a near perfect match (two nucleotide mismatches) with *M. corogypsi*. Although not definitive, the results of the Hoechst stain of the left carpus suggested that there was synovial membrane-associated foreign genetic material within the joint. These foci may have represented mycoplasmal DNA; however, it is possible that this staining represented fragmented heterophil nuclei or other DNA contaminants.

The co-occurrence of synovitis/arthritis, pneumonia, and splenic amyloidosis seems to be compatible with a *Mycoplasma* infection. The lymphoid hyperplasia of the bronchus-associated lymphoid tissue can be interpreted as a classic lesion of mycoplasmosis, although *Mycoplasma* sp. PCR was not performed on the pulmonary lesion. Splenic amyloidosis is evidence of chronic inflammation and can occasionally be seen in mycoplasmosis of poultry with certain strains of *M. synoviae* (Landman and Feberwee, 2001). The histologic appearance of the arthritic change in the Black Vulture in this case was acute, rather than chronic, whereas most articular changes in poultry with *M. synoviae* infection are classically chronic lesions. Specifically, lymphoplasmacytic synovial infiltration, villous synovial hypertrophy, and hypertrophy/hyperplasia of the synovial lining cells have been described in classic mycoplasmal-induced synovitis. None of these findings of chronicity were detected in the examined section of the joint.

Pathogenic mycoplasmas preferentially colonize the mucosa of the respiratory and genital tracts and often establish persistent, superficial infections (Rosenbusch, 1994). Systemic infection and articular involvement can follow this mucosal colonization, especially in immunocompromised hosts (Rosenbusch, 1994). In this case, the concurrent findings of polyarthritis, poor body condition, severe ectoparasitism, and lead toxicosis were interesting, because all indicate a large burden on the immune system. As with all of the pathogenic avian mycoplasmas, the severity of the clinical syndrome may depend on many environmental, agent, and host factors, which makes interpretation of potential predisposing factors in this case difficult (Kleven, 1994).

The role of lead toxicosis, if any, in the poor condition of this bird and in the development of polyarthritis is unknown. Lead ions have a strong affinity for many biochemically reactive compounds and are able to inhibit enzymes involved in major metabolic pathways in many body systems (Locke and Thomas, 1996). Although lead-induced effects on the immune system have not been studied in Black Vultures, there is substantial evidence regarding the negative impacts of lead on immune system function in numerous vertebrate species, as reviewed by Dietert and Piepenbrink (2006). However, there is also evidence that certain species of

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M corogypsi    GCGAATGGGTGAGTAACACGTACTTAACATGCCTTTCAGATTGGGATAACGCTGAGAAAT
VA isolate     GTGAATGGGTGAGTAACACGTACTTAACATGCCTTTCAGATTGGGATAACGCTGAGAAAT
               * **********************************************************

M corogypsi    TAGCGCTAATACCGGATACTTATATTTATCGCATGATAGATATATAAAAGGGGGGGTTTC
VA isolate     TAGCGCTAATACCGAATACTTATATTTATCGCATGATAGATATATAAAAGGGGGG-TTTC
               ************** ****************************************** ****

M corogypsi    CCTCACTGGAAGATTGGGGTGCGTAACATTAGCTAGTTGGTAAGGTAATGGCTTACCAAG
VA isolate     CCTCACTGGAAGATTGGGGTGCGTAACATTAGCTAGTTGGTAAGGTAATGGCTTACCAAG
               ************************************************************

M corogypsi    GCGATGATGTTTAGCGGGGTTGAGAGACTGATCCGCCATACTGGGACTGAGATACGGCCC
VA isolate     GCGATGATGTTTAGCGGGGTTGAGAGACTGATCCGCCACACTGGGACTGAGATACGGCCC
               ************************************** *********************

M corogypsi    AGACTCCTACGGGAGGCAGCAGTAGGGAATTTTCCACAATGGGCGAAAGCCTGATGGAGC
VA isolate     AGACTCCTACGGGAGGCAGCAGTAGGGAATTTTCCACAATGGGCGAAAGCCTGATGGAGC
               ************************************************************

M corogypsi    GACACAGCGTGCAGGAGGAAGGCCTTCGGGTTGTAAACTGCTGTTATAAGGGAAGAAAAA
VA isolate     GACACAGCGTGCAGGAGGAAGGCCTTCGGGTTGTAAACTGCTGTTATAAGGGAAGAAAAA
               ************************************************************

M corogypsi    GTAGGTTAGGAAATGAACTTACCTTGACGGTACCTTGTCAGAAAGCAACGGCTAACTATG
VA isolate     GTAGGTTAGGAAATGAACTTACCTTGACGGTACCTTGTCAGAAAGCAACGGCTAACTATG
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M corogypsi    TGCCAGCAGCCGCGGTAATACATAGG
VA isolate     TGCCAGCAGCCGCGGTAATACATAGG
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FIGURE 5.   ClustalX alignment of the 446-base pair amplicon consensus nucleotide sequence from the Virginia isolate and *M. corogypsi* (GenBank accession L08054) is provided. In comparing the two sequences, there are three nucleotide transitions (G→A, C→T, and T→C) and one nucleotide deletion so that the two sequences share 99.1% identity (*=sequence nucleotide agreement).

vultures tolerate lead exposure to a greater extent than other raptors. Carpenter et al. (2003) found that Turkey Vultures (*Cathartes aura*) may be able to tolerate substantial burdens of lead before overt clinical signs or death occurred, although the controlled study did not examine subtle changes in immune system function. It was not possible to determine whether the bird in this case suffered from lead toxicosis before the manifestation of polyarthritis. Thus, no associations can be made; however, the concomitant findings and the poor overall condition of the bird was interesting.

From 2000 to 2008, six Black Vultures presented to the Wildlife Center of Virginia with arthritis of one or more joints, similar in character to the vulture in this case. Aerobic and anaerobic cultures were performed on three of these cases, all of which exhibited no growth. Furthermore, the arthritis in these three cases resolved with joint lavage and oral enrofloxacin, a drug that shows good efficacy against most mycoplasmas. *Mycoplasma* PCR was not performed in any of these previous cases; however, the similar clinical presentation and response to quinolone therapy is interesting. Conclusive statements regarding the role of *M. corogpysi* as a pathogen of Black Vultures are difficult to make, because this is only the second report of this organism associated with this species (Panangala et al., 1993). Thus, whether *M. corogypsi* is a primary or opportunistic pathogen in free-living Black Vulture populations is unknown.

Future research needs to fulfill Koch's postulates regarding the possible role of *M. corogypsi* as a primary pathogen of Black Vultures and to analyze the epidemiologic importance in free-living populations. Detection of *M. corogypsi* via culture or PCR in samples from asymptomatic Black Vultures obtained by arthrocentesis, tracheal swabs, and air sac biopsies will further the understanding as to whether *M. corogypsi* is an opportunistic commensal organism or a primary pathogen (Lierz et al., 2000).

This case is the first report of a *Mycoplasma* species being found in association with polyarthritis in any species of Old or New World vulture. Furthermore, it represents the first detailed phylogenetic analysis of *M. corogypsi* since the characterization by Panangala et al. (1993).

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