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NOVEL *AVIBACTERIUM* SPECIES ASSOCIATED WITH SINUSITIS AND CONJUNCTIVITIS IN A MERRIAM'S WILD TURKEY (*MELEAGRIS GALLOPAVO MERRIAMI*) FLOCK IN COLORADO, USA

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ABSTRACT: A Merriam's Wild Turkey (*Meleagris gallopavo merriami*) with periocular swelling and periocular skin crusting in Pueblo County, Colorado, USA, was diagnosed with severe catarrhal and fibrinous sinusitis and conjunctivitis. A novel clade of *Avibacterium* was detected in the exudate from this bird. Although eight additional turkeys culled from the affected flock did not have clinical signs or gross lesions, histologically all had mild-to-moderate chronic sinusitis, and infraorbital cultures yielded the same novel clade of *Avibacterium* that was found in the symptomatic turkey. The presence of this *Avibacterium* species in the absence of significant disease in some birds suggested that other factors might have been involved in the development of severe sinusitis and conjunctivitis in the symptomatic Wild Turkey. Negative culture results from a distant flock of Wild Turkeys, acquired with similar methods to the affected flock, suggested that this novel species of *Avibacterium* was not widespread throughout Wild Turkeys in Colorado.

Key words: *Avibacterium*, *Meleagris gallopavo*, *Pasteurellaceae*, sinusitis, wild turkey.

INTRODUCTION

In January 2020, the Colorado Division of Parks and Wildlife (CPW) investigated a report of sick Merriam's Wild Turkeys (*Meleagris gallopavo merriami*) in Pueblo County, Colorado, USA. A landowner had euthanized one sick bird and reported an additional sick bird in the flock. The landowner also noted that the flock of approximately 40 Wild Turkeys had limited contact with her Domestic Pigeons (*Columba livia*), through the hardware cloth fence line of the pigeon enclosure and through access to discarded feed and litter from the pigeons. No other domestic avian species were present on the property. A CPW wildlife officer responded to the report and euthanized one Wild Turkey due to lethargy, periocular swelling, and periocular skin crusting.

Wild Turkey populations are generally stable in Colorado, with no evidence of population-limiting diseases. However, historic concerns for population declines have included disease effects from *Mycoplasma* spp. bacteria (Adrian 1984). Mycoplasmosis can cause upper respiratory diseases such as conjunctivitis and sinusitis that give birds an appearance of periocular swelling (Davidson et al. 1982) and may cause reproductive losses (Rocke et al. 1988). Other diseases that may cause periocular swelling or crusting in Wild Turkeys include avian pox, bacterial dermatitis, and lymphoproliferative disease (Elsmo et al. 2016). One cause of periocular swelling in Domestic Chickens (*Gallus domesticus*) is infectious coryza, caused by the bacterium *Avibacterium paragallinarum* (Anjaneya et al. 2013; Blackall and Soriano-Vargas 2020). Although this disease has not been reported in Wild Turkeys, it is commonly encountered

in backyard poultry (Byarugaba et al. 2007; Clothier et al. 2019). The increasing popularity of backyard flocks in Colorado raises concerns for pathogen transmission between wild and domestic birds. Given concerns for wild and domestic avian health, we investigated the cause of disease in this Wild Turkey flock. We also examined Domestic Pigeons located on the property and Wild Turkeys nearby and distant to the affected flock.

MATERIALS AND METHODS

Sampling of the affected Wild Turkey flock

In January 2020, one Merriam's Wild Turkey with periocular swelling and lethargy was euthanized by gunshot, and the head was submitted to the CPW Wildlife Health Laboratory (CPWWHL) in Fort Collins for postmortem evaluation. After gross examination, we sampled tissues from the eyelid, conjunctiva, and upper respiratory (nasal and infraorbital) sinuses. Exudate from the affected conjunctiva and sinuses was submitted to the Colorado State University Veterinary Diagnostic Laboratory (CSUVDL) for aerobic culture and real-time PCR testing for *Mycoplasma synoviae*, *Mycoplasma gallisepticum* (The National Poultry Improvement Plan 2019), and *Mycoplasma meleagridis* (Raviv and Kleven 2009). Representative tissues were fixed in 10% neutral buffered formalin for at least 48 h. The upper respiratory samples were then decalcified for 48 h with formic acid (CalFor, Cancer Diagnostics, Inc., Durham, North Carolina, USA). Fixed, decalcified tissues were embedded in paraffin blocks, sectioned at 5–8 µm, adhered to glass slides, stained with H&E (all slides) and Gram stain (nasal turbinates), and examined histologically.

In February 2020, eight additional birds from the affected Wild Turkey flock, representing approximately 25% of the total flock, were culled by gunshot. No birds were showing clinical signs, and culling was not selective. The carcasses were donated for consumption, and the heads were transported to the CPWWHL. We examined the heads grossly, aseptically swabbed the infraorbital sinuses, and immediately plated the swabs on Columbia blood (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA) and chocolate agar (Remel, Inc., Lenexa, Kansas, USA) plates for aerobic culture. We collected choanal swabs from the heads postmortem; preserved the choanal swabs in brain heart infusion (BHI) broth (Becton, Dickinson and Company); and submitted samples to the CSUVDL for *M. synoviae*, *M. gallisepticum*, *M. meleagridis*, and avian influenza (Spackman et al. 2002) testing by real-time PCR. Samples from the upper respiratory sinuses and trachea were fixed, decalcified, and prepared for histopathology as described earlier.

Sampling of a connected Wild Turkey flock

To determine the local extent of infection, we opportunistically sampled a second flock of turkeys in Pueblo County in May 2021, approximately 15 km from the original flock and considered a connected population through natural dispersal. Suspected domestic or domestic-wild hybrid turkeys had been identified in the flock based on white plumage and history in the area, and managers intended to remove the suspected domestic birds by culling. Nine birds were trapped by CPW for this management purpose following CPW animal care and use committee capture and handling guidelines. The birds were apparently healthy and were selected for sampling based on presence in the trap concurrent with the targeted white bird(s). We collected blood and two choanal swabs from each captured bird. One choanal swab from each bird was immediately plated on Columbia blood and chocolate agar plates for aerobic culture in the field. A second choanal swab from each bird was placed in BHI transport media. We performed routine testing for health assessments of gallinaceous birds. We submitted choanal swabs to the CSUVDL for *M. synoviae*, *M. gallisepticum*, *M. meleagridis*, and avian influenza testing as described earlier, and we submitted blood from each bird for *Salmonella* Gallinarum and *Salmonella* Pullorum testing by rapid plate agglutination at the CSUVDL.

One of the nine birds captured had all-white plumage and was identified as a probable Domestic Turkey (*Meleagris gallopavo domesticus*). We culled this bird and aseptically swabbed the infraorbital sinuses postmortem for aerobic culture.

Sampling of a distant Wild Turkey flock

In March 2021, we opportunistically sampled eight Wild Turkeys culled from a flock in Archuleta County, Colorado, approximately 400 km from the affected flock. These birds were culled for management purposes in an area where the birds had become a nuisance. Translocation was not a management option due to prior detection of *M. synoviae* in the flock. The birds were captured and euthanized by CPW, following CPW animal care and use committee capture and handling guidelines. The birds were apparently healthy and were selected for culling based on willingness to enter the trap. We collected blood

and choanal swabs before euthanasia. The heads were removed postmortem and the carcasses were donated for consumption. The heads were processed as described for the connected turkey flock, with choanal swabs and blood collected for testing for *Mycoplasma* species, *Salmonella* species, and avian influenza.

Sampling of Domestic Pigeons

The landowner that originally reported the sick turkeys also permitted testing of her 17 Domestic Pigeons that may have contacted the Wild Turkeys. In May 2021, we collected one choanal swab from each pigeon. Each swab was immediately plated on Columbia blood and chocolate agar plates for aerobic culture.

Bacterial classification

Bacterial isolates were screened at the CSUVDL by using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (Bruker MALDI Biotyper microorganism identification system, Bruker, Billerica, Massachusetts, USA). Isolates with similarity scores belonging to genera in the family *Pasteurellaceae* were shipped overnight to the Wyoming State Veterinary Laboratory where a single colony was harvested and passaged onto either a Columbia sheep blood or chocolate agar plate. Inoculated plates were incubated in 5–10% CO_2 at 37 C, until sufficient bacterial growth was observed (after approximately 24–48 h). Bacterial growth was observed for purity. A single colony was selected for MALDI-TOF main spectra profile (MSP) creation and DNA extraction.

MALDI-TOF analysis: Bacteria MSPs were created (see Supplementary Material) by using a MicroFlex MALDI system (Bruker) and software packages (Bruker; see Supplementary Material). The MALDI classification was performed on each created MSP. In addition, MSP dendrogram analysis was performed on a subset of eight representative isolates and included reference MSPs of closely related species: *Avibacterium avium*, *Avibacterium endocarditis*, *Avibacterium gallinarum*, *Avibacterium paragallinarum*, *Avibacterium olantium*, and *Actinobacillus seminis* and outgroup *Pasteurella multocida*.

16S rDNA and whole genome sequencing: For the eight samples examined by MALDI dendrogram analysis, total nucleic acid was extracted, libraries were created, and whole genome sequencing was performed (see Supplementary Material). Genomes were annotated using the Prokka v1.14.6 standard pipeline (Seemann 2014). Genomes were deposited in GenBank as BioProject PRJNA798419 and GenBank IDs SAMN25076281–SAMN25076288 (Supplementary Material Table S1). Complete 16S rDNA sequences were excised from the assemblies of each isolate.

We used BLASTN (National Center for Biotechnology Information [NCBI] 2022) to compare the 16S rDNA sequences from the isolates to the BLASTN 16S rRNA and nucleotide (nt) collection databases. In addition, we compared average nucleotide identify (ANI) by using the whole genome assemblies to all *Pasteurellaceae* species in the NCBI genome database with fastANI v1.32 (Jain et al. 2018).

Phylogenetics: We created a phylogenetic tree based on the core genome of six strains from the *Avibacterium* genus, eight *Pasteurellaceae* isolates from this study, and *P. multocida* as a root. Core genes were discovered and aligned using ROARY v3.13.0 (Page et al. 2015). Genes shared between all strain assemblies were used for maximum likelihood inference by using RAxML v8.2.12 (Stamatakis 2014). We used a generalized time-reversible model with a gamma distribution to account for mutation rate heterogeneity; created 1,000 bootstrap replicates; and performed a search for the best-scoring tree. We evaluated the model for convergence.

RESULTS

Findings from the affected Wild Turkey flock

We found lesions indicating severe sinusitis and conjunctivitis affecting the head of the symptomatic Wild Turkey. Both eyes were swollen shut, and the palpebral skin of the right eye was ulcerated and crusted (Fig. 1A). When the ulcerated skin was removed, this revealed a mat of dense fibrin covering the right eye and extending to fill the infraorbital sinus (Fig. 1B). The right cornea was ulcerated. Fibrin was adhered to the margins of the choanal opening and the commissures of the mouth.

Histologically, the epithelium of the palpebral conjunctiva was attenuated to ulcerated, with goblet cell hyperplasia and adherent pools of mucinous exudate with entrapped cellular debris. The submucosa was diffusely expanded by congestion; edema; and scattered aggregates of subepidermal and perivascular lymphocytes, plasma cells, and fewer heterophils. The nonfeathered skin of the eyelid was regionally ulcerated with an extensive crust of fibrin and necrotic cells (Fig. 2A). The

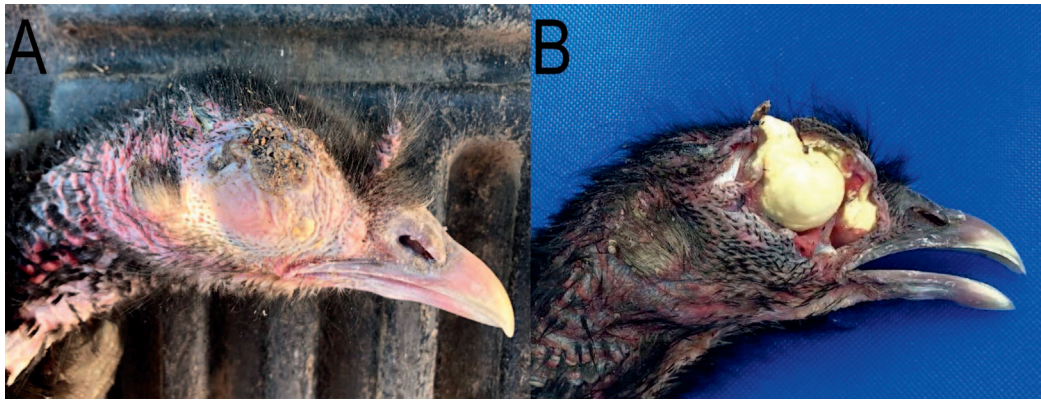

FIGURE 1. Head from a Merriam's Wild Turkey (*Meleagris gallopavo merriami*), case 20-15, submitted January 2020 from Pueblo County, Colorado, USA. (A) Note that both eyes are swollen shut, with ulceration and crusting of the palpebral skin. (B) Removal of the palpebral skin reveals an extensive mat of dense fibrinous exudate covering the right eye and communicating with the infraorbital sinus.

adjacent areas of nonulcerated skin were moderately hyperplastic with orthokeratotic hyperkeratosis. Mats of exudate from the eye and nasal sinuses consisted of lamellated sheets of fibrin and cellular debris with occasional bacterial colonies at the surfaces of the exudate coagula.

The nasal scrolls were irregular and somewhat collapsed, and the overall space within the nasal cavity was moderately dilated. The nasal mucosa was markedly expanded by hyperplastic goblet cells (Fig. 2B) with mild lymphoplasmacytic rhinitis and sinusitis of the inferior nasal meatus and infraorbital sinus. The submucosa was moderately congested and edematous. At the ventral aspect of the nasal cavity, the stratified squamous epithelium showed marked diffuse parakeratosis with gram-negative coccobacilli present at the surface of, and embedded within, the hyperplastic keratin layer (Fig. 2C).

No gross lesions were observed for the heads of eight additional turkeys from the affected flock in Pueblo County. Histologic findings in the upper respiratory tissues from these eight individuals were consistent with chronic mild-to-moderate sinusitis and included mild-to-moderate goblet cell hyperplasia (n=8), mild-to-moderate lymphoplasmacytic rhinitis and sinusitis (n=5), and mild hyperkeratosis (n=3; Fig. 2D). No histologic lesions were observed in the tracheal tissues.

Aerobic culture of exudates from the conjunctiva and sinuses of the affected bird yielded heavy growth of *Pasteurellaceae* bacteria and light growth of *Enterobacter cloacae*. The MALDI-TOF spectra for the *Pasteurellaceae* isolate were adequate for MSP creation. This sample (20-15) was further examined using MALDI dendrogram analysis (Fig. 3), 16S rDNA sequencing, whole genome sequencing, and phylogenetics (Fig. 4). Exudates were PCR negative for *M. synoviae*, *M. gallisepticum*, and *M. meleagridis*.

Aerobic cultures of infraorbital swabs from four of eight turkeys yielded mixed growth or pure growth of a *Pasteurellaceae* species, with one sample having two *Pasteurellaceae* colony types detected. The MALDI-TOF spectra for all five *Pasteurellaceae* isolates were adequate for MSP creation. Three samples (20-126, 20-129, and 20-132) were further examined using MALDI dendrogram analysis (Fig. 3), 16S rDNA sequencing, whole genome sequencing, and phylogenetics (Fig. 4). Choanal swabs preserved in BHI were negative for *M. synoviae*, *M. gallisepticum*, *M. meleagridis*, and avian influenza by PCR.

Results from a connected Wild Turkey flock

No clinical signs or gross lesions were observed in turkeys from the connected Wild Turkey flock in Pueblo County. Choanal

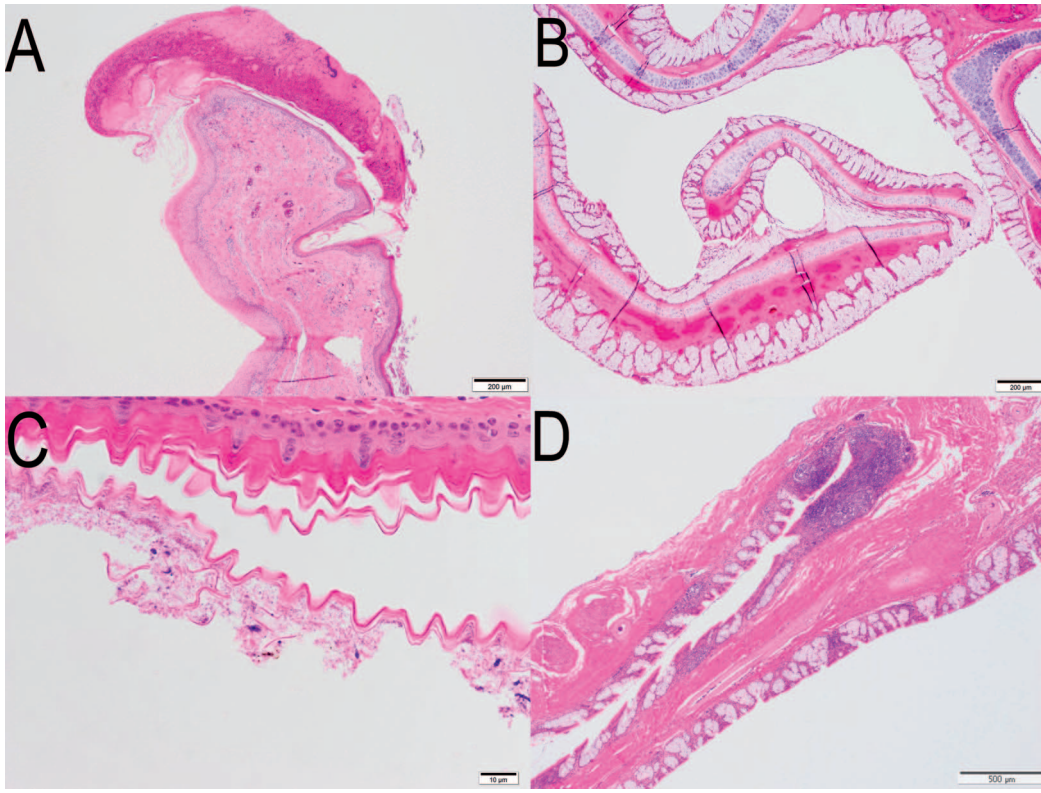

FIGURE 2. Histologic findings from a symptomatic Merriam's Wild Turkey (*Meleagris gallopavo merriami*), case 20-15 (A–C), and an asymptomatic turkey in the flock, case 20-132 (D), submitted January 2020 and February 2020, respectively, from Pueblo County, Colorado, USA. (A) The conjunctival epithelium of the symptomatic turkey is attenuated and covered by an extensive crust composed of fibrin and cellular debris. The subepithelial stroma is expanded by congestion, edema, and lymphoplasmacytic inflammation. (B) The nasal scrolls are irregular and collapsed. Note marked goblet cell hyperplasia. (C) At the ventral aspect of the nasal cavity, the mucosal epithelium shows marked diffuse parakeratosis of the stratified squamous epithelium. Bacteria are present at the surface of, and embedded within, the hyperplastic keratin layer. (D) The submucosa of the inferior nasal meatus of the asymptomatic turkey is moderately expanded by lymphocytes and plasma cells, with mild goblet cell hyperplasia of the mucosal epithelium.

swabs from eight of nine sampled birds grew a *Pasteurellaceae* species in mixed growth. Swabs from the infraorbital sinuses of the culled white turkey grew two morphologically different *Pasteurellaceae* species in light growth and a *Bacillus* species in light growth. The MALDI-TOF spectra were adequate for MSP creation for all nine *Pasteurellaceae* isolates. A subset of the samples (21-595, 21-599, and one isolate from white turkey 21-594) were selected for further analysis using MALDI dendrogram analysis (Fig. 3), 16S rDNA sequencing, whole genome sequencing, and phylogenetics (Fig. 4). Choanal swabs preserved in BHI were negative for *M. synoviae*, *M. gallisepticum*, *M. meleagridis*, and avian influenza by PCR. Blood samples were negative for *S. gallinarum* and *S. pullorum* by rapid plate agglutination.

Results from a distant Wild Turkey flock

No clinical signs or gross lesions were observed in birds from the distant Wild Turkey flock in Archuleta County. No *Pasteurellaceae* bacteria were detected from infraorbital swab cultures, with either no bacterial growth (n=2), light mixed growth (n=4), or heavy mixed growth (n=2) interpreted as normal mucosal flora. Choanal swab pools

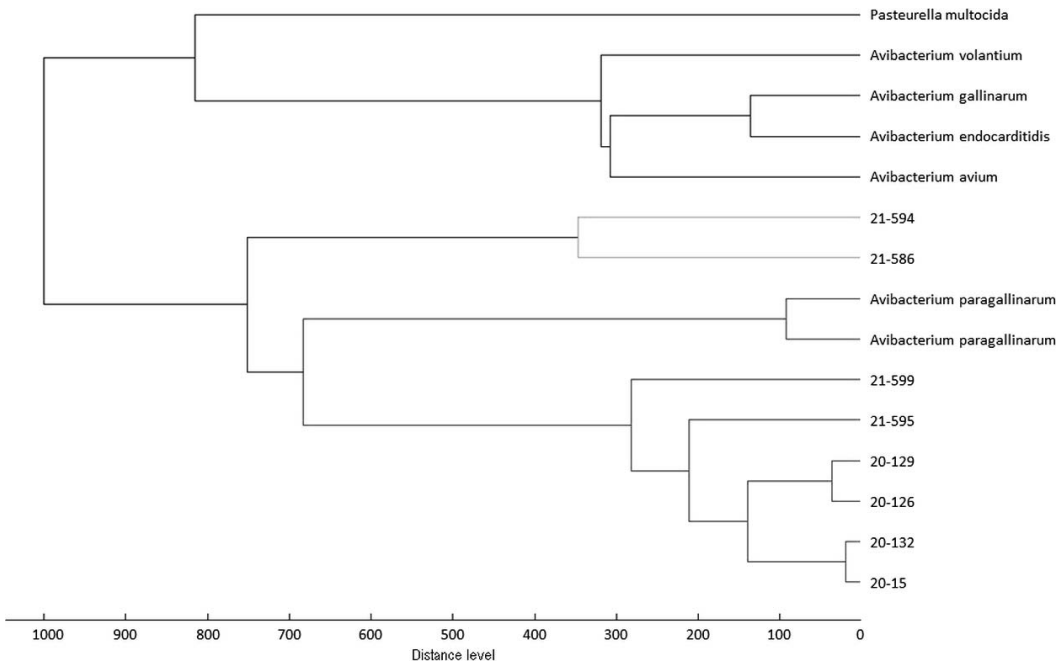

FIGURE 3. The MALDI-TOF main spectra profile dendrogram of *Pasteurellaceae* isolates originating from avian samples from Colorado, USA, 2020–2021. All *Avibacterium* species references provided by the Bruker diagnostic database are included, and *Pasteurella multocida* is used as an outgroup. Isolates from a symptomatic Merriam's Wild Turkey (*Meleagris gallopavo merriami*, 20-15), other Wild Turkeys from the affected flock (20-126, 20-129, and 20-132), and Wild Turkeys from a nearby, connected flock (21-595 and 21-599) cluster as a clade independent from *Avibacterium* references. Associated isolates from a white turkey (21-594) and a Domestic Pigeon (*Columba livia*, 21-586) cluster together independent of the novel turkey clade and *Avibacterium* references but at a greater level than observed for like-species, representing potentially novel unclassified species.

preserved in BHI were positive for *M. synoviae* by PCR, a previous and expected finding in this and other apparently healthy Merriam's Wild Turkeys in Colorado. Histopathology was not included due to the known presence of *M. synoviae* in these birds that prevented interpretation of sinus histology relative to *Avibacterium* species infection. Choanal swabs preserved in BHI were negative for *M. gallisepticum*, *M. meleagridis*, and avian influenza by PCR. Blood samples were negative for *Salmonella* Gallinarum and *Salmonella* Pullorum by rapid plate agglutination.

Results from Domestic Pigeons

No clinical signs or gross lesions were observed in 17 Domestic Pigeons located on the property where the initial symptomatic Merriam's Wild Turkey was detected. A choanal swab culture from one pigeon (21-586) yielded three unique *Pasteurellaceae* colonies in mixed growth, although heavy mixed growth from all of the pigeon choanal swabs may have obscured *Pasteurellaceae* growth. The MALDI-TOF spectra from all three *Pasteurellaceae* isolates were adequate for MSP creation. One isolate was further examined using MALDI dendrogram analysis (Fig. 3), 16S rDNA sequencing, whole genome sequencing, and phylogenetics (Fig. 4).

Bacterial classification

MALDI-TOF analysis: None of the 18 *Pasteurellaceae* isolates from this project with adequate MALDI-TOF spectra for MSP creation could be identified to species (MALDI-TOF scores below 2.0; Supplementary Material Table S1), although scores of ap-

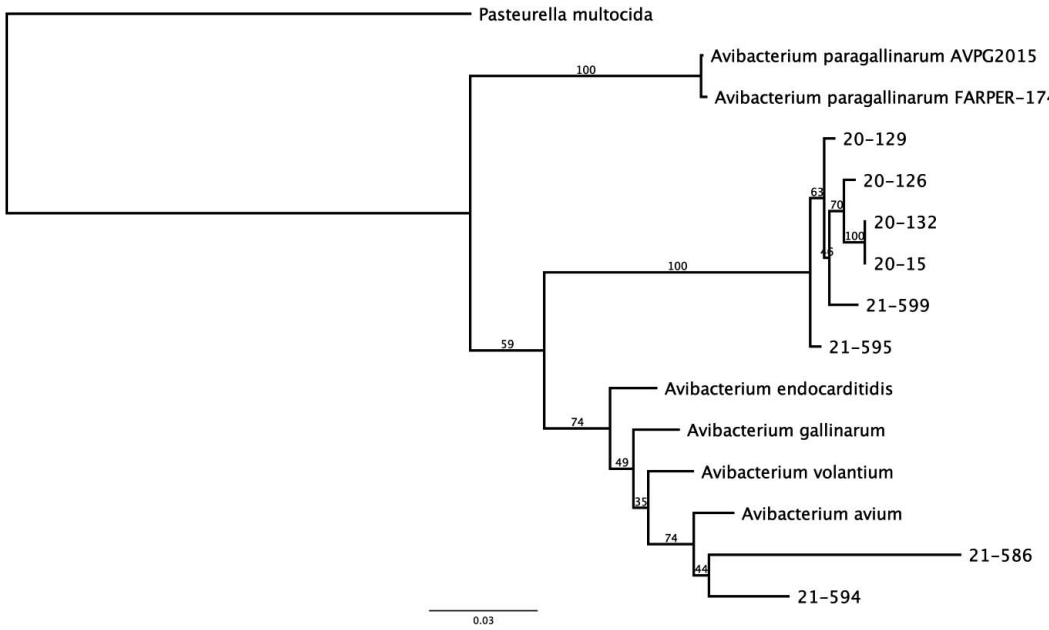

FIGURE 4. Maximum-likelihood phylogeny of *Pasteurellaceae* isolates collected from avian samples in Colorado, USA, 2020–2021, including *Avibacterium* genus reference strains and outgroup *Pasteurella multocida*. Isolates from a symptomatic Merriam's Wild Turkey (*Meleagris gallopavo merriami*, 20-15), other Wild Turkeys from the affected flock (20-126, 20-129, and 20-132), and Wild Turkeys from a nearby, connected flock (21-595 and 21-599) cluster as a clade independent from known *Avibacterium* references. Associated isolates from a white turkey (21-594) and a Domestic Pigeon (*Columba livia*, 21-586) cluster together with *Avibacterium* references, with uncertainty preventing species classification. Bootstrap support values are displayed at the nodes of the tree. Branch lengths are representative of percent patristic distances.

proximately 1.7 suggested genus *Avibacterium*. Several isolates were identified as like-species, with MALDI scores above 2.0 compared with each other. The MALDI dendrogram analysis identified isolates from the symptomatic turkey (20-15), other Wild Turkeys from the affected flock (20-126, 20-129, and 20-132), and Wild Turkeys from the connected flock (21-595 and 21-599) as a clade independent from *Avibacterium* references (Fig. 3). Isolates from the white turkey (21-594) and the Domestic Pigeon (21-586) clustered together but at a distance level greater than distances between *Avibacterium* references, suggesting not like-species to the Wild Turkey clade (Fig. 3).

16S rDNA and whole genome sequencing: The BLASTN (16S rDNA database) hits for the novel Wild Turkey clade included *A. avium* and *A. volantium*, with all hits below the 97% identity like-species threshold (Supplementary Material Table S1). The BLASTN (nt database) hits included *A. paragallinarum* and *A. volantium*, again below the 97% identity threshold. Whole genome ANI analysis of each isolate to all *Pasteurellaceae* species in the NCBI RefSeq database identified *A. gallinarum* as the closest related species, with percent identity <87%, below the like-species threshold. ANI within the novel Wild Turkey clade was >97% identity among the isolates within the novel clade.

The BLASTN (16S rDNA database) hits for the Domestic Pigeon and white turkey isolates included *A. avium* (96.92% identity) and *A. volantium* (97.57% identity), respectively (Supplementary Material Table S1). The BLASTN (nt database) hits included *A. paragallinarum* (97.41% identity) and *A. volantium* (98.45% identity). The ANI comparisons of the pigeon and white turkey isolates to all *Pasteurellaceae* reference genomes resulted in

highest match to *A. avium*, at 90.38 and 95.46% identity, respectively.

Phylogenetics: The Prokka tool predicted a total of 11,644 genes in the eight isolates and seven reference genomes analyzed. It found 45 genes to be homologous (found in all genomes); these were concatenated for each genome and aligned for phylogenetic modeling. We generated 1,000 bootstrap replicates and found convergence after 800 replicates based on a majority rule consensus support of the maximum likelihood tree. The resulting tree depicted references *A. avium*, *A. gallinarum*, *A. endocarditis*, and *A. volantium* as genotypically similar, and distinct from reference *A. paragallinarum*. This is concurrent with *Avibacterium* multilocus sequence type phylogenetic analysis (Bisgaard et al. 2012). Our analysis placed the six isolates representing the novel clade of *Avibacterium* from the Merriam's Wild Turkeys (from the symptomatic turkey and from five asymptomatic birds with histologic lesions) as a monophyletic clade within the *Avibacterium* genus (Fig. 4). The Domestic Pigeon and white turkey isolates aligned most closely with *A. avium*. However, because *Avibacterium* type strains within this clade have been contested as either incipient or misclassified (Bisgaard et al. 2012), no further classifications of these two isolates were performed.

DISCUSSION

Within the *Avibacterium* genus, *A. paragallinarum* is the only known primary pathogen and is the causative agent of infectious coryza, a respiratory disease of domestic chickens (Blackall and Soriano-Vargas 2020). Infectious coryza is a highly contagious disease of the upper respiratory tract affecting broiler and layer flocks worldwide, leading to heavy economic losses (Anjaneya et al. 2013; Blackall and Soriano-Vargas 2020). Typical clinical signs of infectious coryza include nasal discharge, and swelling of the infraorbital sinuses and conjunctiva (Paudel et al. 2017; Crispo et al. 2019; Blackall and Soriano-Vargas 2020). Previous experimental infection studies have indicated that turkeys are among the species resistant to infection with *A. paragallinarum*, and natural infection has not been described in Wild Turkeys (Byarugaba et al. 2007; Blackall and Soriano-Vargas 2020).

It is generally accepted that other Avibacteria (*A. gallinarum*, *A. avium*, *A. endocarditis*, and *A. volantium*) are either opportunistic pathogens or nonpathogenic (Bisgaard et al. 2005; Blackall et al. 2005; Blackall and Soriano-Vargas 2020). Nevertheless, *A. gallinarum* outbreaks have been reported in both chickens and Domestic Turkeys, with a mortality rate of 18–26% reported in turkeys (Bisgaard et al. 2005; Blackall and Soriano-Vargas 2020). *Avibacterium gallinarum* has been associated with at least 14 outbreaks of acute respiratory disease in Domestic Turkeys (Bisgaard et al. 2005). Associated lesions include conjunctivitis, abscesses on the head, airsacculitis, bronchopneumonia, and tracheitis (Bisgaard et al. 2005).

The upper respiratory lesions of the symptomatic Merriam's Wild Turkey described in this study were consistent with other diseases of poultry caused by closely related Avibacteria, and we suspected a causative relationship between the lesions observed and the *Avibacterium* species detected. No other significant bacterial pathogens were detected by aerobic culture, no *Mycoplasma* spp. were detected by PCR, and lesions were not consistent with common diseases of Wild Turkeys such as avian pox or lymphoproliferative disease. The presence of this *Avibacterium* species in other turkeys from the flock in the absence of significant disease suggested that other factors might have been involved in the development of severe sinusitis and conjunctivitis. The presence of this novel *Avibacterium* species in a connected Wild Turkey flock 16 months after detection in the affected flock suggested either maintenance of the bacteria within the population or repeated introductions into the population. Negative culture results from a distant flock of Wild Turkeys suggested that this novel species of *Avibacterium* was not widespread throughout Wild Turkeys in Colorado.

Mass spectrometry and 16S rDNA data indicated that the bacterial isolates from the affected Wild Turkey flock and the connected Wild Turkey flock had greater similarity to each other than to other defined species of *Pasteurellaceae*. Similarity MALDI scores did not identify the species beyond *Pasteurellaceae*. Isolates from the affected turkey flock and nearby connected flock had whole genome ANI values greater than the 95% like-species threshold compared with each other, but below the like-species threshold and above the like-genus threshold (74%) for *Avibacterium* species when compared against the NCBI reference database (Barco et al. 2020). This suggests that the novel clade of isolates recovered in Merriam's Wild Turkeys from Pueblo County, including one bird with severe lesions of sinusitis and conjunctivitis, denotes a novel species within the genus *Avibacterium*. We propose a name for the species: *Avibacterium gallopavo*.

Because the affected flock of turkeys had a history of possible contact with Domestic Pigeons, there was concern for transmission between domestic and wild birds. However, none of the Domestic Pigeon isolates matched the novel clade from the Wild Turkeys, suggesting that the bacteria in the affected flock of Wild Turkeys did not arise from contact with the Domestic Pigeons. The presence of a possible domestic turkey (white feathered) in the Wild Turkey flock supported the suspicion that Wild Turkeys in the area were interacting with domestic birds, but other domestic flocks were not sampled as part of this investigation.

Given the possible role for a novel *Avibacterium* species in lesions of upper respiratory disease in Wild Turkeys, continued monitoring for Avibacteria in wild and domestic gallinaceous birds is warranted. Current disease testing of wild birds in Colorado is driven by translocation activities to address issues of overabundance and to create hunting opportunities. Disease testing protocols for translocation activities are based on the US National Poultry Improvement Plan and are focused on mycoplasmosis and salmonellosis as concerns for domestic and wild gallinaceous birds. Our study suggests a role for Avibacteria in respiratory diseases of Wild Turkeys and suggests that the bacteria are not currently widespread in Wild Turkeys of Colorado. Additional diagnostics including histopathology of additional respiratory tissues, and specific identification of pathogens through immunohistochemistry and PCR, may be useful to help identify associated lesions, better understand the spatial extent of the bacteria, and advise translocation decisions.

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SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/JWD-D-22-00005.

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