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Otitis and meningoencephalitis associated with infectious coryza (*Avibacterium paragallinarum*) in commercial broiler chickens

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Abstract. Infectious coryza, caused by *Avibacterium paragallinarum*, is an acute respiratory disease of poultry that can result in substantial morbidity, mortality, and economic losses. In March 2017, the Turlock branch of the California Animal Health and Food Safety laboratory system encountered an unusual clinical and pathologic presentation of infectious coryza in 6 live, 29-d-old, commercial broiler chickens that were submitted for diagnostic investigation. Antemortem evaluation revealed severe neurologic signs, including disorientation, torticollis, and opisthotonos. Swollen head–like syndrome and sinusitis were also present. Histologically, severe sinusitis, cranial osteomyelitis, otitis media and interna, and meningoencephalitis were noted, explaining the clinical signs described. *A. paragallinarum* was readily isolated from the upper and lower respiratory tract, brain, and cranial bones. Infectious bronchitis virus (IBV) was also detected by PCR, and IBV was isolated in embryonated chicken eggs. Based on sequencing analysis, the IBV appeared 99% homologous to strain CA1737. A synergistic effect between *A. paragallinarum* and IBV, resulting in exacerbation of clinical signs and increased mortality, may have occurred in this case. *A. paragallinarum* should be considered among the possible causes of neurologic signs in chickens. Appropriate media should be used for bacterial isolation, and the role of additional contributing factors and/or complicating agents should be investigated in cases of infectious coryza.

Key words: *Avibacterium paragallinarum*; broilers; meningoencephalitis; otitis.

Infectious coryza, caused by *Avibacterium paragallinarum*, is an acute respiratory disease of chickens, pheasants, guinea fowl, and Japanese quail. This condition has been recognized since the 1930s and can result in substantial economic losses, in both meat-type and egg-type chickens, as a result of poor growth performance, decreased egg production, and increased condemnation rates at the processing plant.[1,2,7] The infection is more commonly observed in intensive poultry farms with multiple age groups, including large-scale egg production and breeding complexes.[2] Traditionally, extensive implementation of all-in, all-out replacement production systems in commercial poultry has resulted in significant resolution of the problem in the broiler industry.[2,7] The commercial layer industry relies mainly on field vaccination for infectious coryza control, most commonly based on the use of autogenous bacterins.[2] The distribution of layer and broiler operations within close geographic proximity represents a significant risk of transmission of *A. paragallinarum* between flocks, particularly in cases of suboptimal biosecurity.[7] Infectious coryza typically occurs as an upper respiratory infection characterized by involvement of the nasal passages and infraorbital sinuses, facial edema, and conjunctivitis. Semi-mature and mature birds appear more severely affected than juveniles, and often exhibit a more prolonged course of the disease. Transmission is usually mediated by contact with symptomatic or carrier birds, airborne droplets, or via contaminated water.[1,2,7] Unusual outbreaks of infectious coryza have been described in commercial poultry production operations in different parts of the world.[1] In particular, a swollen head–like syndrome has been reported in North and South America.[7,16] Septicemia and arthritis have been observed in broiler and layer flocks in Argentina.[16]

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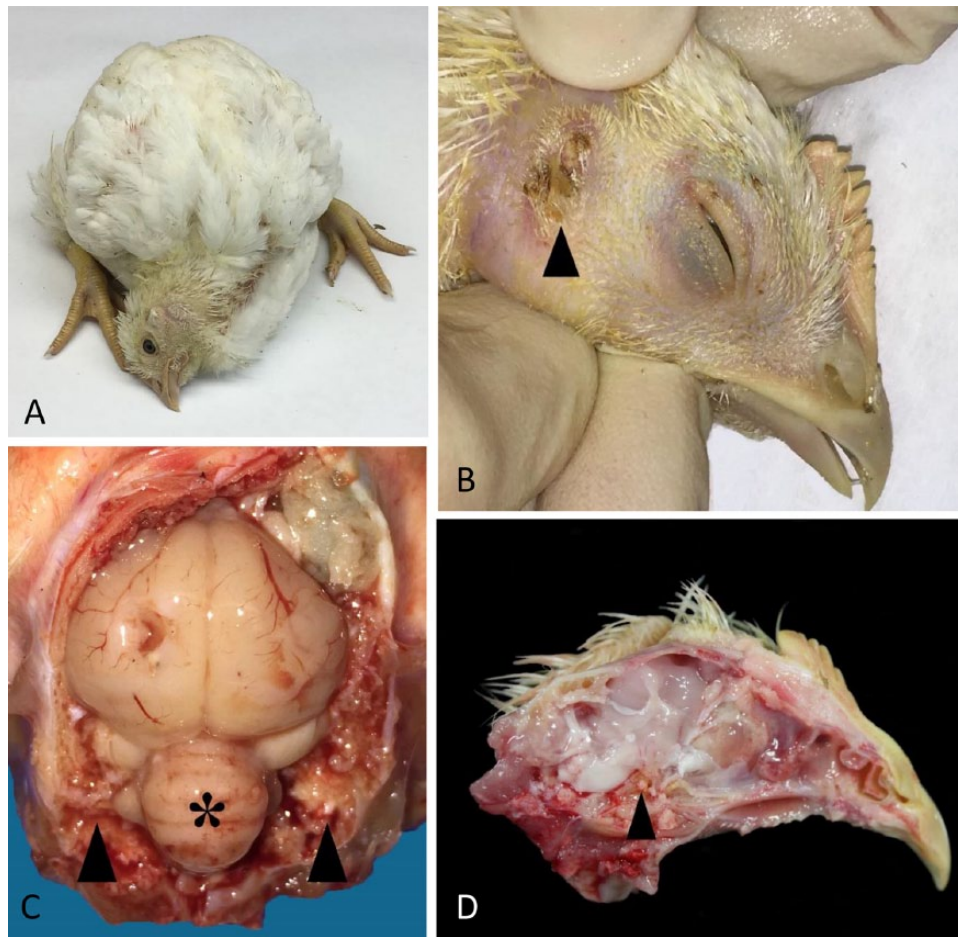
Figure 1. Clinical signs and macroscopic lesions in 29-d-old broiler chickens with otitis and meningoencephalitis associated with *Avibacterium paragallinarum* infection. **A**. Severe torticollis. **B**. External ear. Accumulation of dry, yellow-brown caseous exudate on the auricular feathers (arrowhead). **C.** Cerebral edema and petechial hemorrhages visible on the cerebellum (asterisk), after the removal of the calvaria. Symmetrical discoloration of cranial bones, as a result of accumulation of necrotic exudate, is also present (arrowheads). **D.** Longitudinal section of a head. Focal accumulation of necrotic exudate within a cranial bone (arrowhead).

We describe herein a unique clinical and pathologic presentation of *A. paragallinarum* infection in a group of commercial broiler chickens submitted to the Turlock branch of the California Animal Health and Food Safety (CAHFS) laboratory system. Affected broilers exhibited severe neurologic signs resulting from otitis media, otitis interna, and meningoencephalitis. This case submission was one of 54 cases of *A. paragallinarum* infection received for diagnostic evaluation during an outbreak of infectious coryza involving commercial layer and broiler flocks and backyard chickens in central California in 2017.

In March 2017, the Turlock branch of CAHFS received 6 live, 29-d-old, commercial broiler chickens (Cobb strain) for diagnostic evaluation as a result of a sudden onset of neurologic signs, respiratory distress, and increased daily mortality. Submitted birds originated from a ranch located in central California, comprised of 14 different houses, with ~20,000 birds per house. Morbidity and daily mortality rates were 15% and 1.8%, respectively. All birds were vaccinated against Marek's disease virus (*Gallid alphaherpesvirus 2*) and infectious bursal disease virus (IBDV) using a recombinant vaccine administered in ovo, at 18 d of incubation. A live oocyst coccidiosis vaccine was administered by gel-based application at 1 d of age, at the hatchery. A live modified vaccine for *Salmonella enterica* subsp. *enterica* serovar Typhimurium was also provided at 1 d of age at the hatchery, and at 14 d of age at the poultry house, by gel-based application and drinking water.

Clinical signs included severe disorientation, opisthotonos, and torticollis ($n = 3$; Fig. 1A). Neurologic chickens were restless, unable to stand and walk. Mild-to-severe ocular and nasal discharge, facial edema, and swollen infraorbital sinuses were also present ($n = 2$). The vision of one bird appeared significantly impaired because of palpebral edema and eyelid adhesions resulting from the accumulation of brown dried exudate along the edges of upper and lower eyelids. All birds were euthanized by CO_2, and blood samples were collected from the femoral vein.

At autopsy, infraorbital sinuses contained variable amounts of a mucopurulent white exudate ($n = 3$). Dissecting cellulitis, characterized by subcutaneous gelatinous edema and accumulation of multifocal-to-coalescing yellow dry plaques, was observed in the head and periocular regions ($n = 2$), sometimes extending to the submandibular region, wattles, neck, and thoracic inlet ($n = 1$). Birds exhibiting neurologic signs also had bilateral accumulation of yellow dry exudate on the auricular feathers, almost obliterating the opening of the ear canal (Fig. 1B). Similar exudate was found extending to the external acoustic meatus and invading the air spaces of the cranial bones (temporal and occipital regions; Fig. 1C, 1D). In one bird, petechial hemorrhages were clearly visible on the cerebellum (Fig. 1C) and ocular tissues, including sclera and extrinsic muscles. Congested tracheal mucosa and accumulation of moderate amounts of turbid mucus within the tracheal lumen were present in all 6 birds. One chicken had yellow, caseous exudate within the abdominal air sacs and pulmonary parenchyma.

Skin, brain, cranial bone, infraorbital sinus, air sac, and lung were cultured for aerobic bacteria. Each sample was plated onto 5% sheep blood agar, MacConkey agar, and chocolate blood agar plates, incubated at 37°C and 7% CO_2, and examined at 24 and 48 h post-incubation. *Staphylococcus aureus* nurse cultures were cross-streaked onto both blood agar and chocolate blood agar plates. Smooth, dewdrop, 0.1 mm diameter, cream-colored colonies, compatible with *A. paragallinarum*, were visible on blood agar and chocolate blood agar plates 24–48 h post-incubation. Colonies suggestive of *A. paragallinarum* also exhibited a distinct satellite growth around *S. aureus* nurse colonies, and were confirmed as *A. paragallinarum* by PCR.[5] *A. paragallinarum* was isolated from infraorbital sinus, lung, brain, and cranial bone. No significant bacteria were recovered from skin and air sac.

Sections of skin, eyelid, eye, cranial bone, ear, brain, spinal cord, nasal cavity, trachea, air sac, lung, heart, liver, pancreas, intestine, kidney, spleen, and bursa of Fabricius were collected and fixed in 10% neutral-buffered formalin. All tissues were routinely processed, sectioned at 4-μm thickness, stained with hematoxylin and eosin, and examined by light microscopy. Ear and lung sections were also stained with periodic acid–Schiff (PAS). Nasal passages, infraorbital sinuses, and tracheal sections revealed multifocal-to-diffuse deciliation of the respiratory epithelium, hyperplastic mucus glands, and heterophilic inflammation of the mucosa. Extensive lymphoplasmacytic infiltration of the lamina propria, sometimes associated with lymphoid follicles, was also noticed. The lumen of nasal passages contained a variable amount of mucus mixed with heterophils and exfoliated epithelial cells. Infraorbital sinuses appeared severely distended with abundant fibrinoheterophilic exudate, mixed with rare bacterial colonies. Severe cellulitis, characterized by stromal edema and multifocal sheets of fibrinoheterophilic exudate surrounded by giant cells, was frequently observed within the overlying facial skin, adjacent to the infraorbital sinuses.

Skin sections collected from neck and wattles also revealed diffuse granulomatous cellulitis, partially extending to the muscular layers.

Evaluation of the central nervous system (CNS) revealed moderate-to-severe meningitis with degenerating heterophils and fibrin deposits (Fig. 2A). Inflammation extended to the external layers of cerebellum, cortex, and brain stem, resulting in multifocal hemorrhages, malacic foci, and perivascular cuffing with lymphohistiocytic cells. Heterophilic infiltration of the leptomeninges of the cervical spinal cord was also noticed (Fig. 2B). Temporal and occipital cranial bone sections showed extensive granulomatous reaction associated with marginal multinucleate giant cells, invading the cranial air spaces and extending to the ear. The original architecture of the inner ear was severely distorted (Fig. 2C). In one section, only a few semicircular canals were still recognizable, revealing moderate edema and heterophilic infiltration. Accumulation of keratinized debris, mixed with necrotic exudate was observed within the auditory canal. The tympanic membrane appeared intact, mildly inflamed with heterophils, and a large amount of necrotic exudate was found within the tympanic cavity (Fig. 2D). Ear and cranial lesions were mostly symmetrical. Mild-to-moderate heterophilic infiltration of extrinsic eye muscles and bulbar conjunctiva was also present in a few sections. Additional microscopic findings included multifocal-to-diffuse heterophilic conjunctivitis and blepharitis, airsacculitis, and bronchopneumonia. PAS stain on ear and lung sections did not reveal fungal or yeast elements.

For molecular testing and virology, 2 oropharyngeal and tracheal swab pools and 1 bursa tissue pool were collected at autopsy. The oropharyngeal samples were negative for avian influenza virus (AIV; *Influenza A virus*) and avian paramyxovirus 1 (APMV-1, Newcastle disease virus; *Avian avulavirus 1*), by reverse-transcription real-time PCR (RT-rtPCR) and by reverse-transcription quantitative PCR (RT-qPCR), respectively (National Animal Health Laboratory Network protocol, CAHFS, Davis).[20,23] The tracheal samples were negative for *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) by qPCR (CAHFS, Turlock; IDEXX Laboratories, Westbrook, ME) and positive for infectious bronchitis virus (IBV; *Avian coronavirus*) by RT-qPCR (CAHFS, Davis).[4] IBV was subsequently isolated in embryonated chicken eggs. The allantoic fluid obtained from inoculated eggs was evaluated for the presence of IBV by RT-qPCR targeting the S1 gene of the viral genome; sequence analysis was performed by comparing the consensus strain to known reference strains. Results were presented as percentage similarity to the references strains. The IBV isolated appeared 99% identical to strain CA1737. The bursa of Fabricius, collected in viral transport medium, tested negative for IBDV and very virulent IBDV by RT-qPCR (CAHFS, Davis).[10]

Sera were negative for APMV-1, AIV, IBV, avian reovirus (*Avian orthoreovirus*), and MS by ELISA (IDEXX Laboratories) and negative for MG by plate agglutination
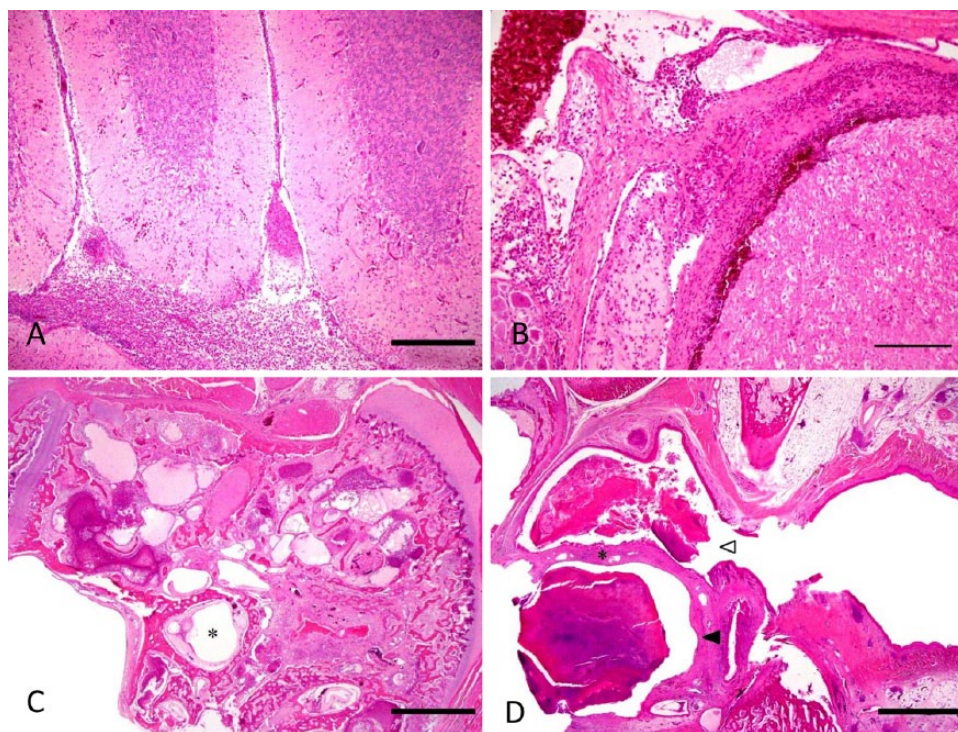
Figure 2. Histologic lesions in 29-d-old broiler chickens with otitis and meningoencephalitis associated with *Avibacterium paragallinarum* infection. **A**. Heterophilic meningoencephalitis of the cerebellum. H&E. Bar = 200 μm. **B**. Heterophilic infiltration of leptomeninges of the cervical spinal cord. H&E. Bar = 100 μm. **C**. Extensive granulomatous osteomyelitis and otitis interna. A semicircular canal is still visible (asterisk), revealing edema and mild heterophilic infiltration. H&E. Bar = 1,000 μm. **D**. Accumulation of keratinized debris and fibrinoheterophilic exudate within the auditory canal (white arrowhead). The tympanic membrane (asterisk) is intact, and a large amount of fibrinoheterophilic exudate is present within the tympanic cavity (black arrowhead). H&E. Bar = 1,000 μm.

test (Charles River Laboratories, Wilmington, MA). Positive antibody titers for IBDV, suggestive of vaccinal immunity, were detected in all 6 birds by ELISA (IDEXX Laboratories).

A. paragallinarum has been isolated from the upper and lower respiratory tract, as well as non-respiratory sites, including liver, kidney, and tarsal joint.[1,2,16] However, we found no previously published reports describing involvement of the CNS and ear with *A. paragallinarum* bacterial infection. The ear in birds consists of 3 compartments within the temporal bone of the skull: the external ear, with the external auditory canal and the tympanic membrane; the middle ear, with the tympanic cavity and the columella; and the inner ear, with the vestibular apparatus and the cochlea. The middle ear is a funnel-shaped tunnel that communicates with the oropharynx, through the Eustachian tube, and with the air spaces of the surrounding bones.[11]

Microscopic evaluation of tissues obtained from the chickens with neurologic signs revealed severe meningoencephalitis and otitis media and interna, with extensive inflammation of the vestibular apparatus and cerebellum, which correlates with the clinical signs observed. Both the ear and brain play a major role in postural equilibrium, spatial orientation, and muscular coordination.[21,22] Otitis media and interna are rarely encountered in avian species. The infectious agents more commonly associated with these conditions include bacteria (*Pasteurella multocida, Riemerella anatipestifer, Escherichia coli, Salmonella* Typhimurium var. Copenhagen and *Salmonella enterica* ssp. arizonae, *Pseudomonas aeruginosa* [Munger LL, et al. *Pseudomonas* infection of the middle ear. Proc 14th Annu Conf Assoc Avian Vet; 1993; Nashville, TN], MG, *Ornithobacterium rhinotracheale*, and *Flavobacterium*),[13,17,18] followed by viruses (APMV-1 and -3, poxvirus, and herpesvirus),[18] and protozoa (*Cryptosporidium baileyi*).[3]

Pathogens can colonize the inner ear following migration from the nasal or oral cavity to the middle ear, through the Eustachian tube (ascending infection).[17] Less frequently, otitis interna can originate from a descending infection from the CNS, via the vestibulocochlear nerve.[12,17] Meningoencephalitis is an uncommon complication of otitis media and interna. Spread of infection to the CNS may occur by erosion of the temporal bone, migration of bacteria along existing vascular or neuronal pathways, or via hematogenous spread.[9] In our case, the most credible hypothesis is of an ascending infection, originating from nasal turbinates and infraorbital sinuses and extending to the middle and inner ear. Gross anatomic changes suggestive of otitis externa were also noticed. Microscopically, although no rupture of the tympanic membrane was identified in the histologic sections examined, focal rupture of the tympanum, with extension of necrotic

exudate through the auditory canal, could not be excluded. Less likely, the otitis externa may have resulted from bacterial colonization of the auditory canal from the skin, following contamination of the auricular feathers by mucoid exudate mixed with bacteria.

The gross anatomic and microscopic findings observed in our birds might also resemble a chronic form of fowl cholera caused by *P. multocida* infection. However, *A. paragallinarum* was the only bacterium recovered from the respiratory tract, cranial bone, and brain, ruling out fowl cholera.

Considering that this flock was not vaccinated for IBV, the isolation of strain CA1737 from the respiratory tract is indicative either of exposure to a vaccine virus circulating in the field or a field challenge. Antibody ELISA for IBV was negative, but this result can occur if birds were submitted before the onset of seroconversion, thus suggesting an acute infection. IBV infection, typically in combination with *E. coli*, is able to induce lesions of facial and periocular cellulitis.[8] A similar synergistic effect between *A. paragallinarum* and IBV, resulting in exacerbation of clinical signs and increased mortality, may have occurred in our case. Several reports hypothesize the role played by other pathogens in unusual outbreaks of infectious coryza, including IBV, MG, MS, *Gallibacterium anatis* biovar *haemolytica, Pasteurella* spp., and *Salmonella* spp.[1,2,7,15,16] Non-infectious factors can also promote the onset of respiratory diseases in poultry.

Adverse environmental conditions, including poor ventilation and excessive ammonia levels, can predispose to viral and/or bacterial colonization of the respiratory epithelium, as a result of detrimental effects on the mucociliary escalator.[6,14] Unfortunately, information on management practices and housing conditions was not available. However, gross or microscopic lesions related to poor environmental conditions, such as corneal erosions and pododermatitis were not detected in submitted birds. In addition, the pathogenicity of *A. paragallinarum* can also vary according to the serovar involved,[19] and further work is in progress in order to characterize the genotype and serotype of the isolate obtained from this case.

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