Pathology in Practice

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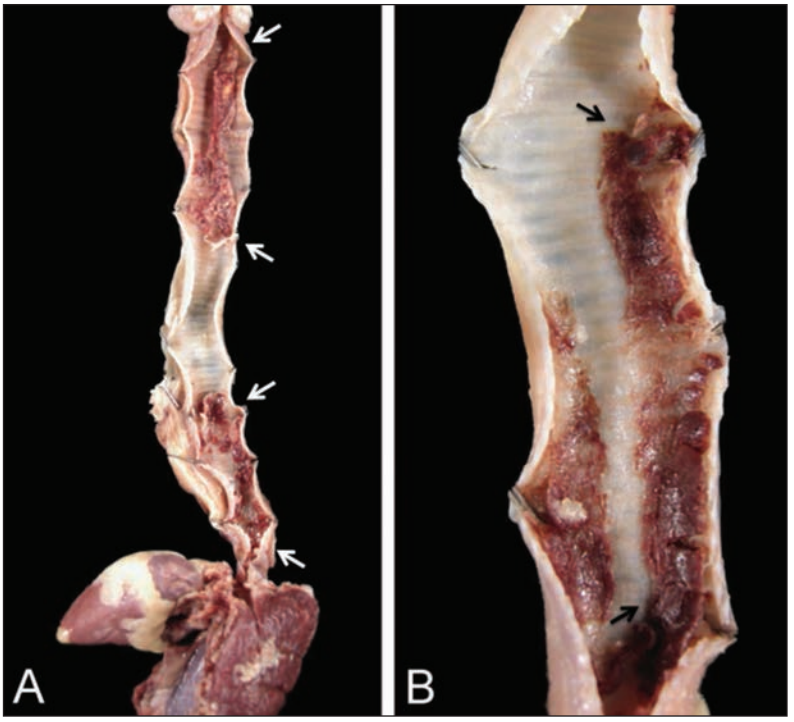

Figure 1—Photographs of the formalin-fixed tracheas of two 18-week-old egg-laying chickens (A and B) found dead on a farm that had a 1-week history of increased mortality rate. The tracheal lumen contains areas of necrotic debris and hemorrhagic exudate (arrows); this exudate was present throughout the length of the trachea in 4 of 5 dead birds submitted for necropsy. Tracheal lesions were absent to minimal in 5 live and apparently unaffected birds from the flock that were euthanized for necropsy.

History

Ten 18-week-old female laying chickens were submitted for necropsy following an outbreak of severe respiratory tract disease that was associated with open-mouthed breathing in affected members of the flock. Five of the birds were found dead, and the other 5 birds had no apparent clinical signs and were submitted live and euthanized with CO_2 just prior to necropsy. The chickens were from a flock of 40,000 birds. The 5 dead chickens were among 2,022 birds in the flock that had died within a 2-week period (5.1% death loss in 2 weeks) in April 2011; acceptable death loss for a 2-week period in that flock was approximately 150 birds (0.4% death loss). The chickens were from an organic farm in Michigan and were unvaccinated. The regular practice on the farm was to move the birds from indoor to outdoor confinement once the ambient temperature was > 15.5°C (60°F). The increased mortality rate occurred during the winter-spring transition when the birds were released from indoor confinement; similar epizootics had occurred on this farm during this transition period annually since 2002, but a diagnosis had not been pursued.

This report was submitted by Sarah A. Hamer, PhD, DVM; Chidozie J. Amuzie, DVM, PhD; Kurt J. Williams, DVM, PhD, DACVP; and Rebecca C. Smedley, DVM, DAVCP; from the Department of Fisheries and Wildlife (Hamer) and the Diagnostic Center for Population and Animal Health and Department of Pathobiology and Diagnostic Investigation, College of Veterinary Medicine (Amuzie, Williams, Smedley), Michigan State University, East Lansing, MI 48824. Dr. Hamer's present address is Veterinary Integrative Biosciences Department, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX 77843.

The authors thank Dr. Ingeborg Langohr and Mohamed Mousa for technical assistance.

Address correspondence to Dr. Hamer (shamer@cvm.tamu.edu).

Clinical and Gross Findings

Within the lumen of the trachea of 4 of the 5 birds that died on the farm, hemorrhagic exudates of varied severity, mixed with sloughed and necrotic debris, were present (**Figure 1**). Tracheal lesions were absent to minimal in the 5 birds that were euthanized. The kidneys in 2 of the birds that had been found dead and in 2 of the euthanized birds were slightly enlarged, pale, and friable with minimal pitting of the cortex. All other organs had no gross abnormalities, and all birds were in good states of nutrition and hydration.

Formulate differential diagnoses from the history, clinical findings, and Figure 1—then turn the page →

Histopathologic Findings

Tissue sections from the heart, lungs, liver, spleen, small and large intestines, and proventriculus of a random subset of the 10 chickens, along with tissue sections of all tracheas and kidneys with gross lesions, were fixed in neutral-buffered 10% formalin and routinely processed for microscopic examination. Within the tracheas of 4 of the 5 birds that had been found dead, large areas of the mucosa were acutely necrotic, with multifocal erosion and ulceration. The remaining mucosa and submucosa were mildly edematous and infiltrated by numerous heterophils, lymphocytes, and plasma cells. The tracheal lumina were filled with fibrinohemorrhagic casts, epithelial syncytial cells, heterophils, and occasional bacteria (**Figure 2**). All the syncytial cells that were observed were sloughed into the tracheal lumina. The nuclei of the syncytial cells had marginated chromatin and often contained single, eosinophilic inclusion bodies (**Figure 3**). Tracheal lesions were absent to minimal in the euthanized birds. In the kidneys, there were patchy areas in which the interstitium was infiltrated with small numbers of mononuclear cells.

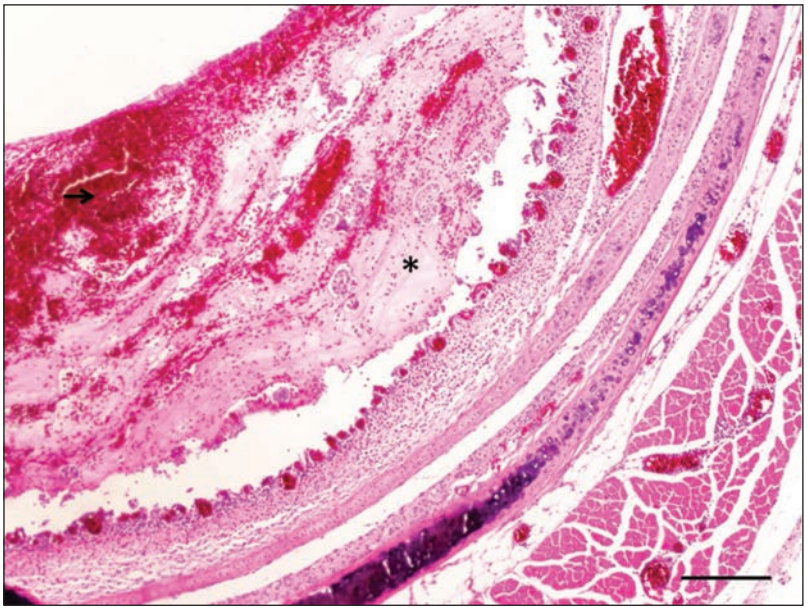

Figure 2—Photomicrograph of a section of the trachea from 1 of the 5 dead chickens submitted for necropsy. A thick exudate of fibrin (asterisk) and hemorrhage (arrow), sloughed epithelial cells, inflammatory cells, and cellular debris are partially occluding the tracheal lumen. The tracheal mucosa is ulcerated and the submucosa is infiltrated with heterophils. H&E stain; bar = 200 µm.

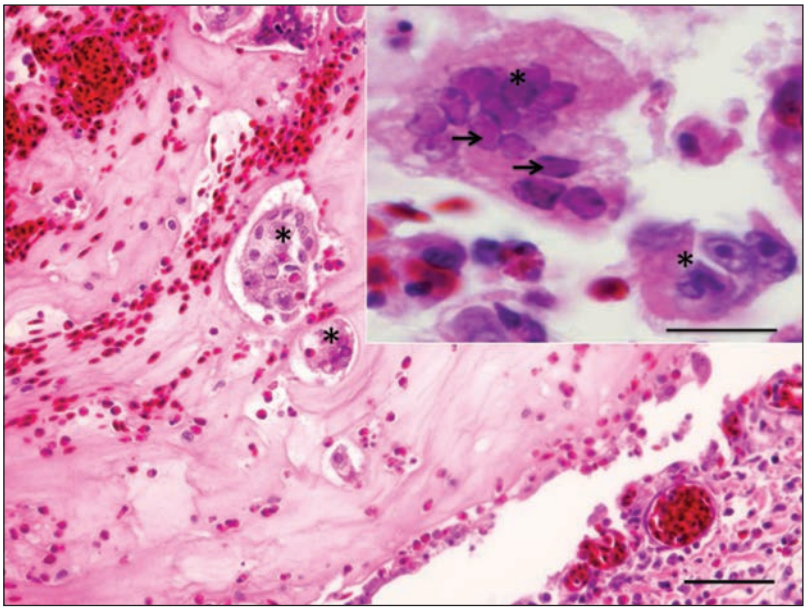

Figure 3—Photomicrograph of a section at the epithelium-luminal interface of the trachea from 1 of 5 dead chickens submitted for necropsy. Fibrinohemorrhagic casts and multiple sloughed epithelial cells (asterisks) are present within the lumen. The mucosa is ulcerated and the submucosa is infiltrated with aggregates of heterophils, lymphocytes, and plasma cells and is mildly edematous. H&E stain; bar = 50 µm. Inset—The sloughed epithelial cells have marginated nuclear chromatin and eosinophilic intranuclear inclusion bodies (arrows). H&E stain; bar = 20 µm.

Laboratory Test Findings

Two pooled samples of tracheal tissues, representative of both dead and euthanized birds, were processed via PCR assay to detect the presence of infectious laryngotracheitis virus, and results were positive. In subsequent genetic analysis performed at the University of Georgia, it was determined that this infectious laryngotracheitis virus was wild type instead of a vaccine strain.

Morphologic Diagnosis and Case Summary

Morphologic diagnosis: Diffuse, fibrinohemorrhagic, and necrotizing tracheitis with syncytial cells and intranuclear inclusion bodies.

Case summary: Infectious laryngotracheitis in laying chickens.

Comments

Infectious laryngotracheitis is a contagious respiratory tract disease that predominantly develops in adult domestic chickens (*Gallus gallus*) as well as other Galliformes, including Indian peafowl, guineafowl, pheasants,[1] and turkeys.[2] The disease is caused by gallid herpesvirus-1 in the genus *Iltovirus* (so named for infectious laryngotracheitis), subfamily Alphaherpesvirinae, family Herpesviridae.[3]

In chickens, infectious laryngotracheitis virus infects the epithelial lining of the upper respiratory tract, causing hemorrhage, necrosis, and ulceration with sloughing of the tracheal epithelium.[4] Damage of the airway causes severe tracheal rales and bloody discharge from the beak and nostrils, with death attributed to suffocation or secondary invasion by bacteria or fungi.[1] Transmis-

sion occurs through the upper respiratory tract, ocular route, and ingestion. Infection also reduces weight gain and egg production in chickens, thereby causing severe economic losses to the poultry industry.[5] Infectious laryngotracheitis virus strains vary in virulence, and some infections become latent, during which time the viral genomic material is incorporated into the host DNA. Stressful events, such as mixing of flocks or the commencement of egg laying, may induce viral shedding into the trachea; that viral shedding can become the source of new outbreaks in flocks.[6] The in ovo route of transmission has not been demonstrated for infectious laryngotracheitis virus.[7] Humans may serve as fomites and transmit the virus between flocks when biocontainment protocols are not followed.

Identification of the infectious laryngotracheitis virus can be achieved by viral isolation from tracheal swabs or the trigeminal ganglia (the trachea and trigeminal ganglia being the main sites of latency for the virus[8]); identification of virus via electron microscopic examination of tracheal exudate or tracheal epithelial scrapings; detection of viral antigens through immunofluorescence testing, agar gel immunodiffusion assays, ELISAs, or PCR assays; or microscopic demonstration of intranuclear inclusion bodies in the epithelial cells of the trachea or in the sloughed cells within the lumen of the trachea.[9] Infectious laryngotracheitis intranuclear inclusion bodies are the Cowdry type A inclusions of herpesviruses but may only be present for 3 to 5 days after infection.[9]

Infectious laryngotracheitis is currently among the 14 avian diseases listed with the World Organization for Animal Health (OIE) and was considered a list B disease under a previous classification system. List B diseases are defined as transmissible diseases that are of socioeconomic or public health importance within countries and that are important in the international trade of animals and animal products.[10] The disease has a worldwide geographic distribution and is found in most countries with commercial poultry-raising operations. For control of infectious laryngotracheitis, chickens can be immunized with attenuated live virus vaccines. These vaccines can be delivered via eye-drops, aerosols, or drinking water. However, modified-live vaccines possess variable degrees of residual virulence, which may increase with serial passage among birds and can cause disease in flocks.[6]

The flock described in the present report was located on an organic farm in Michigan. Michigan Department of Agriculture guidelines outline 3 management options when infectious laryngotracheitis is diagnosed on a farm: depopulation, vaccination, or nonintervention.[11] The depopulation option involves a quarantine of the newly infected flock; depopulation of the entire flock; cleaning and disinfection of all contaminated equipment, cages, and buildings under the supervision of Michigan Department of Agriculture staff; and maintenance of quarantine for a minimum of 2 weeks following depopulation. The vaccination option involves the use of chick embryo vaccination to help halt the spread of infection within the flock and requires approval from the state veterinarian. This management intervention involves quarantine, cleaning, and disinfection of the entire premises; vaccination of all birds in the flock; and release of quarantine only after a period of at least 2 weeks during which there is no death loss from respiratory tract disease or any clinical signs of infectious laryngotracheitis in the flock. The nonintervention option involves quarantine of the entire premises for a minimum of 2 weeks and release of quarantine only after all necessary cleaning and disinfection has occurred and a minimum period of 2 weeks during which there are no signs of infectious laryngotracheitis in the flock. As a result of the diagnosis in the case described in the present report, the farm manager elected to pursue the vaccination option. The farm was allowed to maintain organic status as specified under the livestock requirements of the USDA National Organic Program.[12] Given that the main mode of transmission of infectious laryngotracheitis virus is through the respiratory route and is facilitated by close contact among chickens, this case illustrates that outbreaks may also occur in cage-free, outdoor settings, coincident with the seasonal transition of chickens from an indoor to outdoor environment.

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