Clostridial Infection of the Proventriculus and Ventriculus in Broiler Breeder Pullets

Thomas A. Gaydos Jr.*, Sabrina Hurst-Proctor*, Susan Williams†
*Gaydos Technical Services, LLC † The University of Georgia

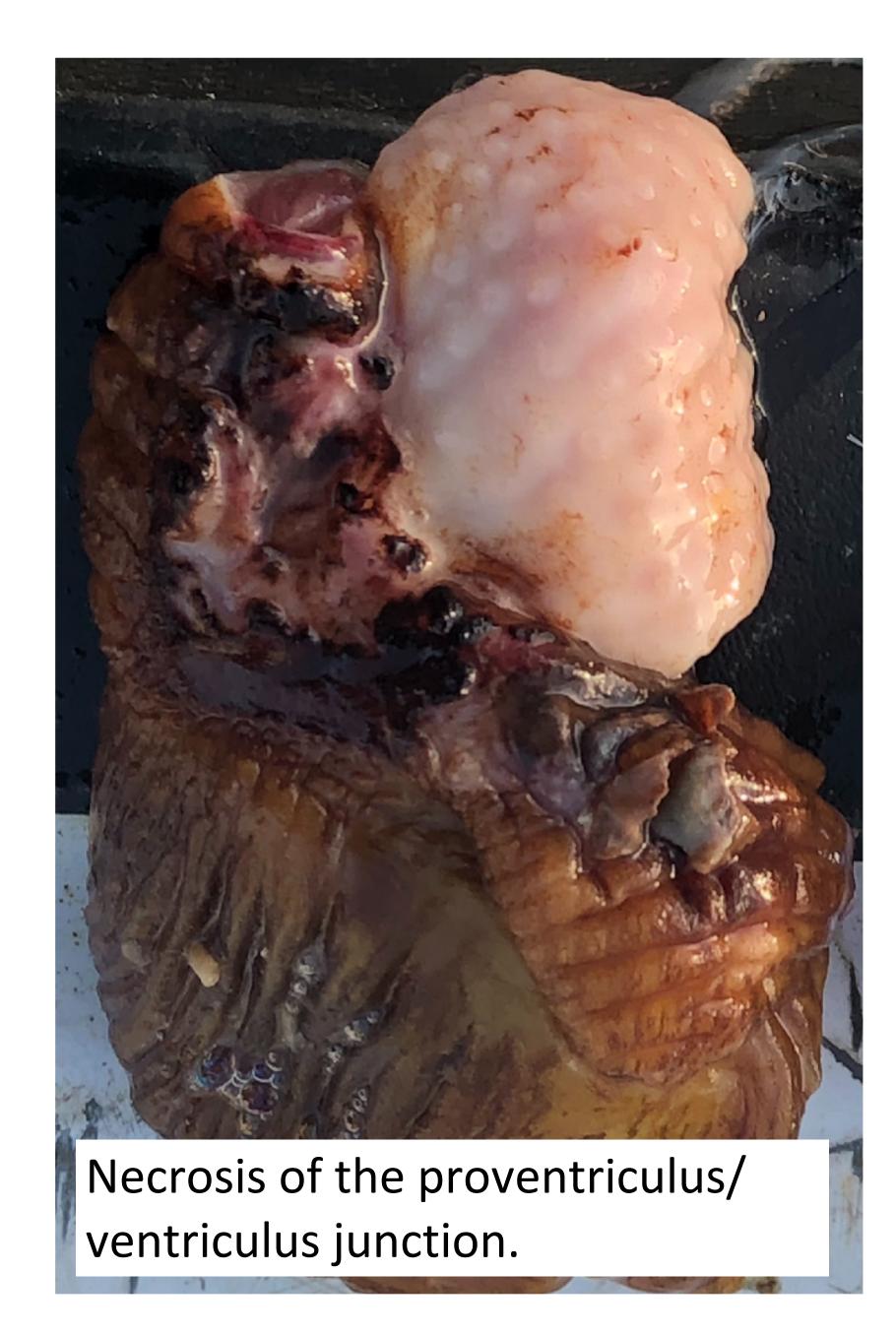
Abstract

Several broiler breeder pullet flocks of varying ages at a single company had increased mortality. The service technicians noticed a correlation between mortality and significant gross gizzard lesions. The technicians noted that copper sulfate administration in the water reduced the mortality. A flock of 18 week old 308AP pullets with elevated mortality was identified to have lesions consistent with the reports of the field technicians. On gross examination, the gizzard lesions were similar to lesions reported by the technicians; severe ulcerations were noted at the junction of the proventriculus and ventriculus. Gizzards were collected for histopathology and anaerobic microbiology. *Clostridium perfringens* was isolated from the gizzard, and gram positive and negative bacteria were noted in and around the lesions utilizing Brown and Hopps stain. The *C. perfringens* was alpha toxin positive and netB negative by PCR. Meat and bone meal was a suspected cause of contamination; therefore meat and bone meal samples from the feed mill were submitted for anaerobic culture. Several of the samples were positive for *C. perfringens* and *C. bifermentans*. The *C. perfringens* was positive for alpha toxin and negative for netB. Changes were made to meat and bone meal processing and delivery; the incidence of the lesions was significantly reduced in the complex.

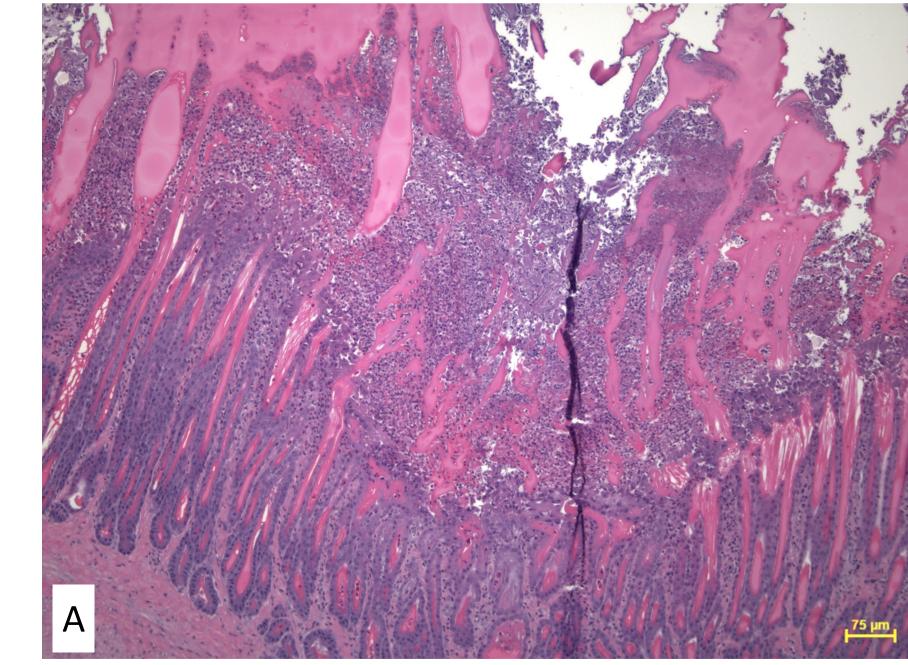
Microbiology and Molecular Results

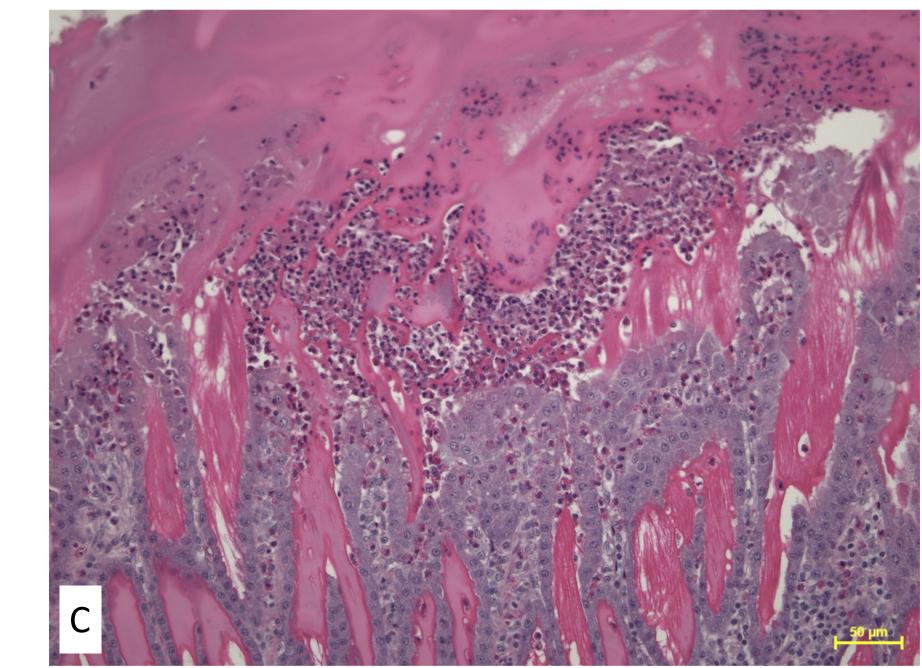
Sample		C. perfringens	α-toxin	netB toxin
Gizzard House 1		2/2	2/2	0/2
Gizzard House 2		4/4	1/1	0/1
Meat and Bone Meal	Clos	stridium	α-toxin	netB toxin

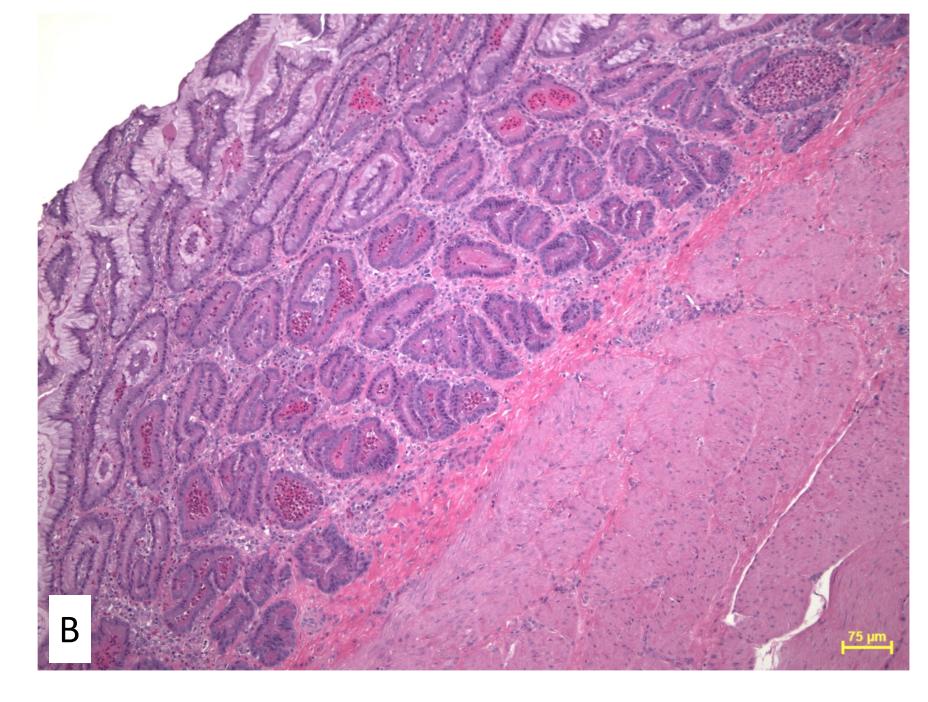
GIZZAI A I I GAS		- / -	
Meat and Bone Meal Sample	Clostridium	α-toxin	netB toxin
Truck 80	1/1 C. perfringens	1/1	0/1
Truck 86	1/1 C. bifermentans		
Bin 6	1/1 C. perfringens	1/1	0/1
Bin 6	1/1 C. perfringens 1/1 C. bifermentans 1/1 C. barattii	Not tested	Not tested
Auger 6	1/1 C. perfringens	1/1	0/1
Auger 7	1/1 <i>C. perfringens</i> 1/1 <i>C. difficile</i>	1/1	0/1



Gross and Microscopic Appearance







- **A.** Ventriculus. The mucosal layer with koilin has cell debris and there is acute necrosis of the villi epithelial cells. H&E
- **B.** Ventriculus/proventriculus junction. The crypts are filled with heterophils at the junction of the ventriculus/proventriculus. H&E
- **C.** Ventriculus. The villi lamina propria has mild heterophilic infiltrates and the koilin has necrotic cells and degenerate heterophils. H&E

Conclusion

The severe lesions in the proventriculus and ventriculus contained gram-positive, rod-shaped bacteria. The same samples were culture positive for several species of *Clostridium*. In a continuation of the investigation, several meat and bone meal samples also tested positive for Clostridium species. Both the ventriculus and the feed ingredient samples were positive for α-toxin positive, NetB toxin negative *C. perfringens*. The prevalence of a toxin-producing *C. perfringens* in both meat and bone meal and the ventriculus is suggestive of the ingredient as a source. *Clostridium perfringens* is a causative agent for necrotic enteritis in chickens. *Clostridium bifermentans* is generally considered non-pathogenic, but has been rarely recovered from human infections. *Clostridium baratti* is associated with botulism in humans. *Clostridium difficile* is commonly isolated from the intestines of many species. It can be asymptomatically carried but can be a cause of severe diarrhea and colitis in humans. More work is needed to prove Koch's postulate, but there was a link between the inclusion of contaminated feed ingredients and the lesions.

References

Barrett LF, Saragadam SD, DiMaria CN, Delgado-Daza A. Infection of a prosthetic knee joint with *Clostridium bifermentans*. Oxf Med Case Reports. 2020 Aug 10;2020(8):omaa057. doi: 10.1093/omcr/omaa057. Czepiel J, Dróżdż M, Pituch H, Kuijper EJ, Perucki W, Mielimonka A, Goldman S, Wultańska D, Garlicki A, Biesiada G. Clostridium difficile infection: review. Eur J Clin Microbiol Infect Dis. 2019 Jul;38(7):1211-1221. doi: 10.1007/s10096-019-03539-6. Epub 2019 Apr 3.



Harvey SM, Sturgeon J, Dassey DE. Botulism due to Clostridium baratii type F toxin. J Clin Microbiol. 2002 Jun;40(6):2260-2. doi: 10.1128/JCM.40.6.2260-2262.2002. Swayne, D. E. (20130716). Diseases of Poultry, 13th Edition [VitalSource Bookshelf version]. Retrieved from vbk://9781118719732