

## Black proventriculus in Cobb 500 broiler breeders

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### Abstract

The black proventriculus is a necropsy finding sign observed during post-mortem examinations of dead young chicks up to 10 days of age and due to variation in color of the affected proventriculus organ, it may be ignored in some cases, in particular when the disease occurs simultaneously with other well-recognized infections of two weeks old chicks. In late January 2018, several live and dead chicks with a history of an unusual increase in mortality showing no clinical signs but sudden death were referred to the poultry clinic of Faculty of Veterinary Medicine, Urmia University, Urmia, Iran. General routine postmortem examination revealed color changes (light green to dark green color) in the wall of proventriculus together with some degrees of congestion in the lungs and liver of the dead chicks. Microbiology tests revealed that the causative agent was *Escherichia coli* strain O142 and histopathological examination indicated that the color changing of the organ was due to necrosis of glandular epithelium with locally extensive or diffuse hypercellularity by lymphocytes and macrophages with hemorrhages. In conclusion, black proventriculus due to *E. coli* strain O142 was responsible for the sudden death of young chicks and based on antibiogram, selective antibiotics should be used for the treatment of the affected flocks.

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### Introduction

The black proventriculus is caused mainly by O142:H avian pathogenic *Escherichia coli* (APEC) that was first reported in young broiler breeder chicks in 2015.<sup>1</sup> The O142 proventricular isolate is highly pathogenic, 100-fold higher than those of other tissues' isolates, via air sacs than via oral routes, indicating that the major transmission route of the black proventriculus is respiratory tract rather than the digestive tract.<sup>2</sup> Gastroenteritis due to *E. coli* O142 in human<sup>3</sup> and *E. coli* O142 induced diarrhea in animals<sup>4</sup> have been reported. However, documented data on black proventriculus caused by *E. coli* O142 are limited, therefore, this clinical report may contribute to the routine diagnosis of black proventriculus in young chicks.

### Case Description

All procedures and chickens handling were carried out according to the standard animal experimentation

protocols of the Veterinary Ethics Committee of Faculty of Veterinary Medicine, Urmia University (Urmia, Iran).

In late January 2018, an unusual increase in mortality was reported in a pen of one of the premises of a broiler breeder farm with a capacity of 35,000 birds and seven premises in West-Azerbaijan province, Iran. The percentage of mortality rate from day 1 to day 10 was 0.06, 0.09, 0.09, 0.75, 0.97, 1.45, 1.97, 2.24, 2.83, and 0.36 respectively. Daily mortality was increased from the third day and on the age of 6<sup>th</sup> day, some live and dead chicks were referred to the clinic, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran. The highest mortality (2.83%) was recorded on the 9<sup>th</sup> day of age. During the time, 6<sup>th</sup> day of age, we visited the poultry farm, the chicks of affected premises and the others did not show any specific clinical symptoms, and only dead chicks (1.45%) were observed. Examination of the dead and live chicks were carried as recommended<sup>5</sup> and based on necropsy findings, primarily the disease was diagnosed as black proventriculus and the samples of

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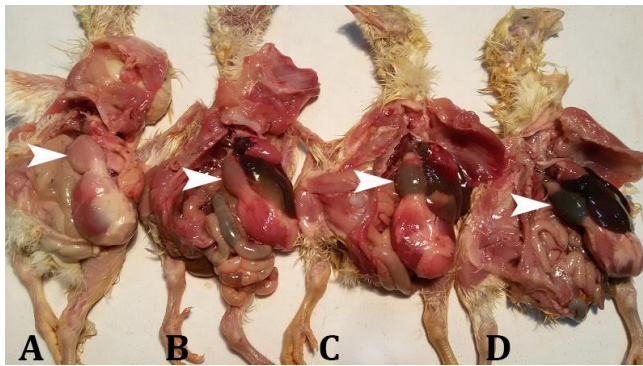


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affected organs including proventriculus, lung, liver, and heart) were obtained for histopathology and microbiology assessments.<sup>5</sup>

## Results

During necropsy examination, an indication of color changes including light green to dark green in the proventriculus (Figs. 1 and 2) of the dead chicks as well as some degrees of congestion in the lungs and liver of the dead chicks were observed. Based on microbiology results, the causative agent was diagnosed *E. coli* (Microbiology Laboratory, Faculty of Veterinary, Urmia University, Urmia, Iran) and serogrouped as O142 strain by the microbiology section of Razi Institute (Karaj, Iran). In addition, histological examination showed necrosis of glandular epithelium with locally extensive or diffuse hypercellularity by lymphocytes and macrophages with hemorrhages that all together created the color changing of the proventriculus (Fig. 3). Histological changes in other organs were also presented in Figure 4.

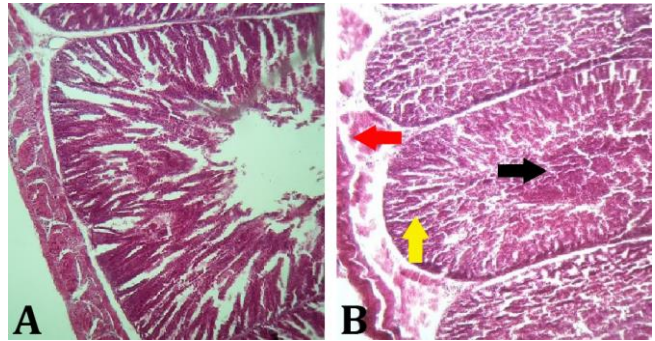


**Fig. 1.** Proventriculus color (serosal surface). **A)** Normal proventriculus. **B, C, D)** Black proventriculus. The color changes varied from light to dark green in different intensities in the proventriculus. The liver and lungs were congested.

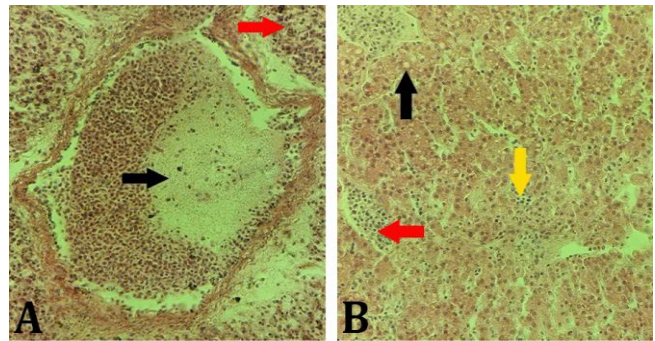


**Fig. 2.** Proventriculus color (epithelial surface). **A)** Normal proventriculus and **B)** Black proventriculus.

General recommendations were given based on colibacillosis and a selective antibiotic (++++) prescribed based on the results of the antibiogram.



**Fig. 3.** Photomicrograph of the proventriculus. **A)** Normal proventriculus and **B)** Black proventriculus in which the mucosa is eroded with fibrinonecrotic membrane, focal areas of necrosis, and hemorrhage (yellow arrow). Proventriculus glandular lumen is filled with hemorrhage and cell debris (black arrow), edema, and hemorrhage in the muscular layer proventriculus (red arrow), (H & E, 100 $\times$ ).



**Fig. 4.** **A)** Photomicrograph of lung indicating severe pulmonary congestion and edema with marked infiltration of mononuclear cells in the lung and bronchi (red arrow). The vessel was dilated with thrombosis (black arrow). **B)** Photomicrograph of liver indicating severe hemorrhage (red arrow), fatty degeneration (black arrow), and necrosis with infiltration of inflammatory cells (yellow arrow), (H & E, 100 $\times$ ).

## Discussion

Proventriculus anatomically is located between crop and ventriculus (gizzard), histologically it consists of four layers of the mucous, submucosa, tunica muscularis, the serosa, and physiologically it secretes mucus, hydrochloric acid, and pepsinogen.<sup>6-9</sup> Among diseases of the proventriculus, black proventriculus is the latest one that has been recently reported from broiler breeders for the first time in 2015.<sup>1</sup>

Lack of clinical signs as well as no mortality in broiler breeders during 1-3 days of age observed in this study was in agreement with previous reports.<sup>1,2</sup>

Regarding green/dark blue/black discoloration of the proventriculus (Figs. 1 and 2); a) it has been reported that the characteristic features of colisepticemia at

necropsy are tissues that develop a green discoloration following exposure to air possibly related to the indole produced by the bacterium<sup>1</sup> and this may explain why black proventriculus is almost observed in autopsied dead rather than alive chicks, b) APEC strain O142 isolated from affected proventriculus carry the *feoB* gene,<sup>2</sup> *feoB* gene is a GTPase and has a role in ferrous iron transport,<sup>10</sup> therefore, the presence of ferrous iron (FeO) may contribute to greenish discoloration (green-blue/blue-dark, based on the pH) of the proventriculus in the affected chicks.<sup>11</sup>

The causative agent (*E. coli* O142) was isolated in this study agreed with previous reports.<sup>12</sup> Regarding breed specificity of black proventriculus as stated by Wang *et al.*,<sup>1</sup> some questions should be answered including a) Arbor Acres (AA) and Cobb strains are not a breed and they are considered as strains while AA is one of the Aviagen brands but Cobb 500 is one of the Cobb-Vantress brands, b) *E. coli* O142 has been isolated from different poultry breeds such as broiler breeders<sup>1</sup> as well as layer breeders<sup>12</sup> in which breeds of the layer are different from breeds of the broiler. However, black proventriculus diagnosed in this clinical report was seen in Cobb 500 broiler breeder was in agreement with the previous reports<sup>1</sup> and could contribute to the idea that this brand may be more susceptible to black proventriculus (APEC O142) as previous reports showed some degree of susceptibility among genetically different poultry breeds to colibacillosis.<sup>13</sup>

Antibiotic susceptibility tests indicated that APEC O142 strains are highly resistant to some routinely used drugs,<sup>1,14</sup> however, excellent activity of fosfomycin against *E. coli* isolates including fluoroquinolone-resistant strains was observed during this study and our results agreed with the previous report.<sup>15</sup> In conclusion, black proventriculus should be considered as a new form of pathological manifestation of *E. coli* infections in chickens, and antibiotics with +++ sensitivity are recommended as used in this case.

### Conflict of interest

The authors declare no potential competing conflict of interest.

### References

1. Wang X, Cao C, Huan H, et al. Isolation, identification, and pathogenicity of O142 avian pathogenic *Escherichia coli* causing black proventriculus and septicemia in broiler breeders. *Infect Genet Evol* 2015; 32:23-29.
2. Wang XB. Identification of avian pathogenic *Escherichia coli* causing black proventriculus of broilers and the biological characterization of its OmpF and MetF Genes. PhD Thesis. Basic Veterinary Science. Yangzhou University, China: 2017.
3. Gerards LJ, Hennekam RCM, Dijk WCv, et al. An outbreak of gastroenteritis due to *Escherichia coli* O142 H6 in a neonatal department. *J Hosp Infect* 2016; 5(3): 283-288.
4. Zahraei Salehi T, Safarchi A, Rabbani Khorasgani M. Identification of virulence genes in isolated *Escherichia coli* from diarrheic calves and lambs by multiplex polymerase chain reaction. *Pak J Biol Sci* 2006; 9(2): 191-196.
5. Collett SR. Principles of disease prevention, diagnosis, and control. In: Swayne DE (Ed). *Diseases of poultry*. 13<sup>th</sup> ed. Ames, USA: Wiley-Blackwell 2013; 3-40.
6. Nasrin M, Siddigi MNH, Masum MA, et al. Gross and histological studies of digestive tract of broilers during postnatal growth and development. *J Bangladesh Agril Univ* 2012; 10(1): 69-77.
7. Svihus B. Function of the digestive system. *J Applied Poult Res* 2014; 23(2): 306-314.
8. Denbow DM. Gastrointestinal anatomy and physiology. In: Scanes CG (Ed). *Sturkie's avian physiology*. 6<sup>th</sup> ed. UK: Elsevier 2015; 337-366.
9. Zhang H, Ge T, Peng S, et al. Microstructure features of proventriculus and ultrastructure of the gastric gland cells in Chinese taihe black-bone silky fowl (*Gallus gallus domesticus* Brisson). *Anat Histol Embryol* 2016; 45(1): 1-8.
10. Lau CKY, Krewulak KD, Vogel HJ. Bacterial ferrous iron transport: the Feo system. *FEMS Microbiol Rev* 2016; 40(2): 273-298.
11. Wei H, Donohoe BS, Vinzant TB, et al. Elucidating the role of ferrous ion cocatalyst in enhancing dilute acid pretreatment of lignocellulosic biomass. *Biotechnol Biofuels* 2011; 4: 48. doi:10.1186/1754-6834-4-48.
12. Bautista BC, Venice Ann Lorreine M, Mendoza BC. Serogroup, pathotype and multiple drug resistance of *Escherichia coli* strains isolated from the cloaca of layer chickens in San Jose, Batangas, Philippines. *Philipp Sci Lett* 2018; 11: 69-77.
13. Ask B, van der Waaij EH, Stegeman JA, et al. Genetic variation among broiler genotypes in susceptibility to colibacillosis. *Poult Sci* 2006; 85(3): 415-421.
14. Elsayed ME, Shabana II, Esawy AM, et al. Detection of virulence-associated genes of avian pathogenic *Escherichia coli* (APEC) isolated from broilers. *J J Genetics* 2017; 2(1): 004.
15. Ko KS, Suh JY, Peck KR, et al. *In vitro* activity of fosfomycin against ciprofloxacin-resistant or extended-spectrum beta-lactamase-producing *Escherichia coli* isolated from urine and blood. *Diagn Microbiol Infect Dis* 2007; 58(1): 111-115.