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Anatomical Pathology and Histology of the Trachea and Lungs of Broiler Infected with *Avibacterium paragallinarum*

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ABSTRACT

This case study aims to determine the causes of pathological changes in anatomy and histology in the trachea and lungs of the broiler. This study was taken from a broiler of Mr Alif Husbandry, who had clinical symptoms of clear discharge from sinuses, anorexia, conjunctivitis and difficulty breathing on July 5th 2022, in Gresik. The anatomical pathology and histological examination results showed changes in the trachea and lungs, showing hemorrhage and inflammatory cell infiltration. The results of microbiological examination from the sinus swab identified *Avibacterium paragallinarum*. In this study, clinical symptoms, anatomical pathology, and histology of the trachea and lungs of broiler chickens were caused by infection with *Avibacterium paragallinarum*.

Keywords: Anatomical Pathology, Histology, Trachea and Lungs, Broiler, *Avibacterium paragallinarum*

INTRODUCTION

Infectious coryza (IC) or snot is an upper respiratory tract infection that affects chickens. This disease is caused by *Avibacterium paragallinarum*, previously known as *Haemophilus paragallinarum* (Cigoy et al., 2016). The clinical signs are nasal discharge, conjunctivitis, facial swelling, lacrimation, and anorexia. Generally, the clinical symptoms can last for 2 to 3 weeks. However, as *A. paragallinarum* is a conditional pathogenic bacterium, the severity of clinical signs depends on age, breed, and factors such as poor feeding management, parasitism, and mixed infections (Guo et al., 2022).

Significant economic losses due to IC include increased culling, decreased egg production (10-80%), decreased body weight, stunted growth, and some deaths (2-10%). This disease can be found worldwide, especially in tropical countries. Diagnosis can be based on a history of rapid disease spread, clinical symptoms, and pathological changes caused by snot (Wahyuni et al., 2018).

A. paragallinarum is a Gram-negative, polar-staining, non-motile bacterium. In 24-48 hrs cultures, it appears as short rods or coccobacilli 1-3µm in length and 0.4-0.8µm in width, with a tendency for filament formulation. The organism degenerates within 48-60 hrs, showing fragments and indefinite forms (Yamamoto, 1991; Akter et al., 2013). IC had been diagnosed based on a postmortem examination of dead birds, but no attempt was made to isolate the causal agent of IC, *A. paragallinarum*. Therefore, this investigation was undertaken with a view (a) to isolate and identify the etiological agent of IC, *A. paragallinarum*, from layer chickens and (b) to determine the pathological lesions in affected organs (Akter et al., 2013).

This study describes the anatomical pathology and histology changes in the trachea and lungs of a broiler infected with *Avibacterium paragallinarum*.

MATERIALS AND METHODS

This study of case report was conducted on July 5, 2022, on a broiler at Alif Animal Husbandry, which is located at Jl. Dewi Sekar, Gresik. A broiler is female; it has anorexia, so, at the age of ± 3 months old, it just has a 500-gram body weight. The broilers are kept in cages with a population of 50 broiler chickens, with drums made of wood, dirty environment, and unvaccinated. The history of the broiler chickens includes clear discharge from sinuses, anorexia, conjunctivitis (inflammation of the lining of the eye), and difficulty breathing.

The broilers were euthanized and tested ethically at the Research Ethics Commission of the Faculty of Veterinary Medicine, Universitas Wijaya Kusuma Surabaya (Certificate Number: 124-KKE). Broiler was subjected to necropsy and observation of anatomical pathology in the respiratory organs (trachea and lungs).

The abnormalities of the trachea and lungs were prepared with Haematocilin Eosin

staining to determine the histological changes. Swabs from sinuses are used for the isolation and identification of bacteria. The results of the anatomic pathology, histology and microbiology (bacteria) were then analyzed descriptively.

A swab from the sinuses with a sterile cotton swab was then cultured on blood agar media using the quadrant method and incubated for 24 hours at 37°C with anaerobes. Bacterial colonies are identified by observing colony growth characteristics and bacterial cells' characteristics through gram staining. Bacterial identification was continued with the TSIA test containing glucose, lactose, sucrose, peptone, sodium thiosulfate, and phenol red SAC. Methyl Red-Voges Proskauer (MR-VP) test, which contains peptone, glucose and phosphate buffer, Simmons's Citrate Agar (Oxoid), which contains sodium citrate, ammonium phosphate and bromthymol blue, Sulfide Indole Motility (SIM) which contains peptone from casein, peptone from meat, ammonium iron citrate, sodium thiosulfate. Then, all the tubes were incubated at 37°C for 18-24 hours. Indole was added with Kovac's reagent, MR with 5-10 drops of methyl red solution, and VP with KOH and α-naphthol.

RESULTS AND DISCUSSION

The clinical signs of broiler chickens include clear discharge from sinuses, anorexia, conjunctivitis (inflammation of the lining of the eye), and difficulty breathing. The broiler has a necropsy. The results of the anatomic pathological examination of the trachea showed bleeding petechiae lesions on the mucosa and wet mucosa; on histological, there was desquamation of the cynociliary columnar epithelium on the mucous layer, the presence of hemorrhage on the mucosal layer, and the presence of inflammatory cell infiltration on the mucosal layer.

The results of the anatomical, pathological examination of the lungs showed congestion and light bleeding. On histology, there was congestion of blood vessels, hemorrhage in several places, atelectasis and emphysema in parabronchi, and inflammatory cells.

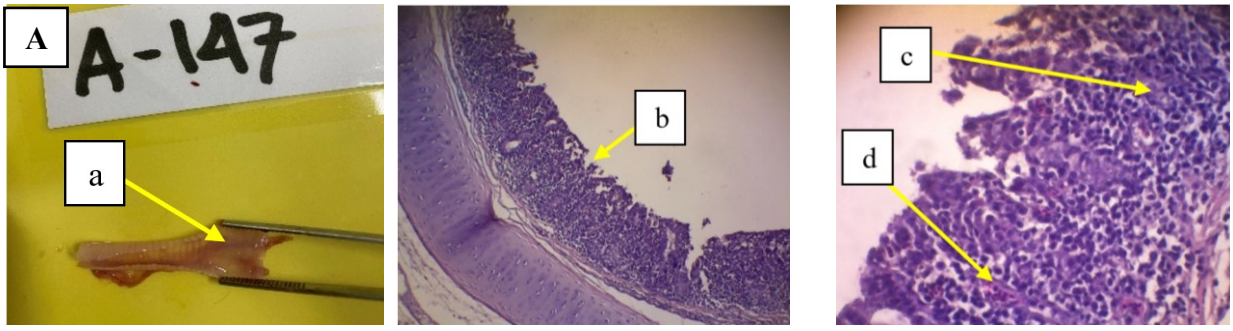

Figure 1. A. Trachea. Description: a). Haemorrhagic petechiae lesions on the mucous layer looked wet on the tracheal mucosa. b). Desquamated epithelial tissue (HE 100x), c). Inflammatory cell infiltration of the epithelium, d).Haemorrhagic lesions of the epithelial lining (HE 400x).

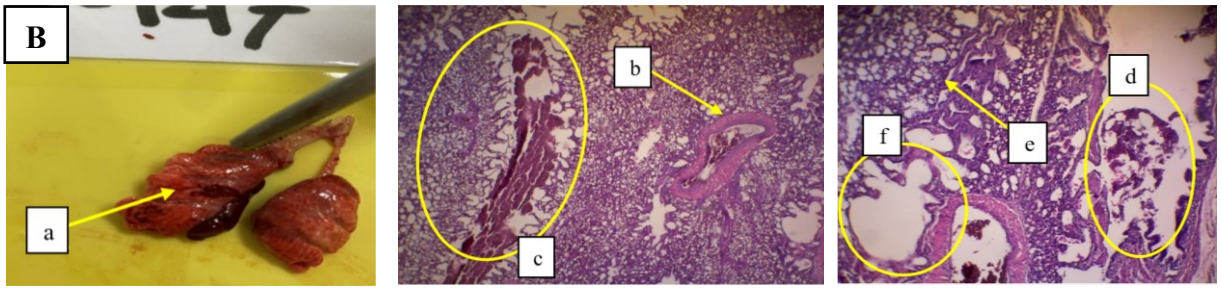

Figure 2. B. Lungs. Description: a. congestion (parabroncus), b. haemorrhage petechiae, c. tissue congestion (HE 40x), d). desquamated tissue, e) and f). atelectasis and emphysema of the alveoli (e,f) (HE 100x).

Infraorbital sinus swabs are collected and streaked on blood agar. The plate was incubated at 37°C for 24–48 hours. Biochemical tests like Indole, Methyl Red, Voges-Proskauer, Citrate, Oxidase, Catalase and sugar fermentation tests were carried out to identify isolates according to the methods described.

Small, round, grey-white colonies were detected in Blood Agar after 24 hours of incubation—gram stain on organisms expressing Gram-negative and coccobacillus. The organisms found were oxidase-negative, catalase-negative, negative H2S, nitrate and glucose fermentation reduction, lactose, sucrose and mannitol but not galactose. Based on morphological and biochemical characteristics, the organism was identified as *Avibacterium paragallinarum* (Beiranvand et al., 2022; Akter et al., 2014).

The mucous membrane of the trachea is composed of a ciliated pseudo-complex epithelium with a few goblet cells. Cilia play a role in the defence mechanism of the avian respiratory system in cooperation with goblet cells. Ciliary activity will push antigens or foreign bodies captured by the mucus into the pharynx. Loss of cilia will interfere with cilia's movement, making chickens susceptible to disease. The epithelium that makes up the trachea has a vital function in the defense of the respiratory tract, so structural damage or desquamation of the epithelium can affect the quality of air entering the lungs. Epithelial desquamation is the detachment of the epithelial layer on the mucosal tissue. Desquamation of epithelial cells can be caused by the damaging properties of pathogenic agents or by repairing damaged epithelial layers due to pathogen infection.

Inflammatory cells found in these three layers are neutrophils. This study/case is caused by *Avibacterium paragallinarum*, so they are dominated by neutrophil inflammatory cells. Neutrophil cells are the first white blood cells to migrate from blood vessels to sites of inflammation. The function of neutrophils is to phagocytize bacteria and cellular debris. Neutrophils also release chemicals that attract other white blood cells to the site of inflammation by chemotaxis. Inflammation is a response of the body's defence mechanism against damage that affects tissues, both local and those that enter the body.

Hemorrhage is characterized by bleeding from the blood vessels due to endothelial damage. Erythrocytes from the blood vessels are broken down quickly and phagocytosed by macrophage cells around the inflamed tissue. This microscopic appearance was also found in the histopathological picture of the trachea of a sample of chickens with snot symptoms (Figure 1. A). Pathogenic agents that enter the body will respond quickly to the body's defence system through inflammation mechanisms. Inflammation will be preceded by an increase in vascularity, which results in the number of cells in the blood vessels increasing, and if this continues, the blood vessels will rupture. Hemorrhage will occur (Milo et al., 2019).

Bacteria can cause disease in several mechanisms, one of which is the production of toxins in the form of endotoxins and exotoxins, which can produce various pathological effects. The toxin in *Avibacterium paragallinarum* increases blood circulation organs, followed by an increase in pressure in the vascular (Ali et al., 2013). As a result, the lungs experience congestion. Congestion is the accumulation of blood in the veins due to blood flow slowing down or even stopping. General congestion involves the circulation to both the liver and lungs. General congestion can be fatal or cause death. In this study of vascular congestion in the lungs, according to the study of Singh et al. (2013) and Dwivedi et al. (2018), lesions such as congestion, desquamation of epithelial cells and infiltration of mononuclear cells can cause blockage, bleeding, thickening of the interlobular septa and infiltration of mononuclear cells in the alveolar walls in the lungs.

Emphysema is characterized by permanent enlargement of the parabronchial breathing space and damage to its walls (O'Dowd, 2020). Furthermore, according to Haryo (2021), lung emphysema is a condition in which the lungs are enlarged, caused by excessive swelling of the alveoli accompanied or without tearing the alveoli walls depending on alveoli damage. Air is present in the cavities of the interstitial tissue or remains in the alveolar cavities only. The process can run acutely or chronically. In general, emphysema of the lungs is characterized by expiratory dyspnea, hyperpnoea and easy fatigue, as seen in the symptoms in broilers in this study. Breathing is challenging due to swelling in the infraorbital, which causes the chickens to have difficulty breathing (dyspnea). Furthermore, in the case of atelectasis, the condition of atelectasis is the condition of the collapse of the alveoli. Collapsed alveoli do not contain air, so they cannot participate in gas exchange.

CONCLUSION

Anatomical pathology and histological changes in the trachea of broilers infected with *Avibacterium paragallinarum* include bleeding

petechiae lesions on the mucosa and wet mucosa, desquamation of the cynociliary columnar epithelium on the mucous layer, the presence of hemorrhage on the mucosal layer, and the presence of inflammatory cell infiltration on the mucosal layer. The lungs showed congestion, light bleeding, congestion of blood vessels, hemorrhage in several places, atelectasis and emphysema in parabronchi, and inflammatory cells.

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