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Histopathology of Esophagus and Crop of Pigeon (*Columba livia*) Infected by *Trichomonas gallinae*

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ABSTRACT

Background: *Trichomonas gallinae* is a protozoa that causes trichomoniasis which is common found in pigeons and it attacks the digestive tract of pigeons. Proper examination can be done to differentiate from healthy pigeons.

Purpose: to observe the changes in histopathological images of the esophagus and the crop of pigeons (*Columba livia*) that infected with *Trichomonas gallinae*.

Method: Observations were carried out on 20 pigeons with symptoms of presence *Trichomonas gallinae*. Swab examination in the esophagus with the Natif Test and Giemsa Staining. Esophagus and crop was collected through necropsy in the upper digestive tract. Then histopathological examination was performed with Hematoxylin Eosin (HE) staining. Data was analyzed descriptively.

Result: Histopathological features of pigeons (*Columba livia*) infected with *Trichomonas gallinae* were different from healthy pigeons. Pigeons infected with protozoa *Trichomonas gallinae* showed changes of the esophagus in the form of erosion and cell necrosis in stratified non-keratinized epithelium (mucosa) and infiltration of inflammatory cells in the mucosa layer and sub esophageal mucosa. In addition, changes in the crop were cell necrosis in the mucosa layer, infiltration of inflammatory cells in the submucosal layer, and congestion in the sub mucosal cavity.

Conclusion: There were histopathological changes in the esophagus and crop of pigeon that infected with *Trichomonas gallinae*.

Keywords: Histopathology, Esophagus, Crop, *Trichomonas gallinae*, pigeon.

Introduction

Pigeon (*Columba livia*) is one type of fowl with a well known species in the Columbidae family. It is one of the fauna diversity in Indonesia which has a high diversity of phenotypes, both qualitative and quantitative characteristics.¹ Pigeons are also farmed for their meat as a source of animal protein. The problem is poor maintenance management causes pigeons become susceptible to disease.² One of parasitic infection that can attack a pigeon is *Trichomonas sp.*³ *Trichomonas*

gallinae is a flagella protozoa that causes trichomoniasis in pigeons.⁴ *Trichomonas gallinae* is most commonly found in pigeons and has been reported to infect 67.3% of pigeons species.⁵ Trichomoniasis causes high mortality in young pigeons and affect the pigeons mortality with a percentage of 77.5% in one subpopulation.^{6,7} *Trichomonas gallinae* attack the digestive tract of pigeons, mostly found in the oral cavity, pharynx, esophagus, and crop of adult pigeon as carriers of the disease.⁸

Trichomonas gallinae infection has a pathognomonic clinical signs. The identification can be done from taking samples of lesion in the mouth both nichrotic and fluid lesion. Transmission can occur through milk from parent and child of pigeons, as well as through contaminated food and beverages. This infection can spread between

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species such as bird and eagle, then considered risk for human health. Species that infect birds and domestic mammals have been identified in human clinical samples. Several phylogenetic analyzes have identified trichomonas derived from animals as taxa of closed relatives of two species of humans.⁹ This confirms that efforts to detect disease in pigeons are an important to improve an animal health while distinguishing infected pigeons from healthy pigeons. To reduce the risk of zoonosis more effectively from industrial food animal production, the application of biosecurity must be in harmony with the interest of public health.¹⁰ Surveillance of diseases, as well as in transmission model for poultry and zoonotic diseases are the main things.¹¹ In addition, the most important is rapid detection and diagnosis.¹²

Observation of symptom is part of the framework of detection and diagnosis. Pigeons infected with *T. gallinae* will have clinical symptoms namely weight loss, weakness, depression with lesion in the upper gastrointestinal tract.³ Infection *Trichomonas gallinae* causes ulceration in the crop, esophagus and proventriculus. Microscopic examination of pigeons infected with *Trichomonas gallinae* will show an infiltration of heterophil and eosinophil cells in the mucosal layer and submucosal lamina propria.¹³ Parasitic infection *Trichomonas gallinae* can cause infected birds have clinical symptoms that similar to avian fowl pox, deficiency of vitamin A, poultry (fowl diphtheria), capillariasis, candidiasis and aspergillosis, namely the presence of necrotic lesions or lesions in the esophagus. *Trichomonas gallinae* infection can be established by identification of the *T. gallinae* parasite in the esophageal swab examination. The method to determine the presence of *Trichomonas gallinae* is by examining the esophagus swab. Esophageal swab is conducted by the negative method to detect the movement of the parasitic *Trichomonas gallinae* flagella. Giemsa staining is used for morphological identification of *Trichomonas gallinae*.⁵

In the course of this disease, the difference from other diseases that have similar clinical symptoms can be used as guidelines for management according to the disease found. The information about certain organs changes in pigeon will open up the opportunities for the virus schemes spreading. This study aimed to determine the histopathology of the esophagus and crop in pigeons infected with *Trichomonas gallinae*.

Method

This study was an an observational study. Laboratory examination were conducted at the Parasitology Laboratory, Department of Veterinary Parasitology, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya Indonesia for 20 weeks. The preparation of esophageal and crop histopathological preparations was conducted in the Department of Veterinary Pathology, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya Indonesia. This study used replacement, reduction and refinement. Animals were modified in research procedures in such a way that to reduce pain and stress in experimental animals, and considered to the 5 principles of freedom in animal prosperity.

A total of 20 pigeons (*Columba livia*), both male and female contributing to the sample with the criteria have clinical symptoms of *Trichomonas gallinae* infection. The symptoms were depression, weight loss, hair standing like cold and gloomy color, when standing curled up, there were lesions with wet and sticky exudates in the oral cavity, especially in acute diseases and in chronic infections there are hard exudations.

Sample examination was carried out in several stages. The first step was a swab examination in the esophagus through Natif tests and Giemsa staining when the pigeons were still alive. The samples were positive if found *Trichomonas* parasites, proven by the movement of flagellars from *Trichomonas gallinae* with a 100-400 times magnification microscope. Pigeons with a lesion in the esophagus were positively infected with *Trichomonas gallinae*. Esophagus and crop collecting was done through necropsy in the upper digestive tract, then were given formalin 10%. After that making histopathological preparations with Hematoxylin Eosin (HE) staining for microscopic examination. Data of changes in esophagus histopathological histories and crop was analyzed descriptively.

Result

Total of 20 pigeons (*Columba livia*) with clinical symptoms of *Trichomonas gallinae* infection were positively infected with *Trichomonas gallinae*. Histopathological changes in the esophagus and crop will be seen as a decrease in blood flow (congestion), irreversible tissue necrosis, partial loss mucosa (erosion), and cells with a defensive reaction as a

response to injury in the form of a vascular reaction to infection (inflammatory cells). Changes in oral lesions and esophagus swab results that showed infection with *Trichomonas gallinae* are shown in figure 1.



Figure 1: Lesions in the oral cavity (A), esophagus infected with *Trichomonas gallinae* (B).

The result of the esophagus swab examination with the natif method was obtained by the movement of flagella from the parasite *Trichomonas gallinae*. Protozoan was detected from the motility or movement of flagella and in Giemsa staining will leave a bluish color up to purple. The movement of flagella *Trichomonas gallinae* was found in all samples of pigeons' esophagus.

Based on the results of examination and microscopic observations carried out there was a histopathological change in the esophagus of pigeons infected with *Trichomonas gallinae*. These changes

in histopathological features included erosions of cell mucosa necrosis due to *Trichomonas gallinae* infection. Description of histopathological changes in the esophagus of pigeons (*Columba livia*) infected with *Trichomonas gallinae* which has erosion and cell necrosis can be seen in figure 2.

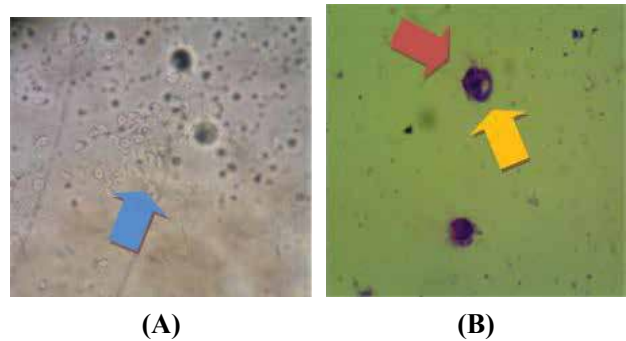


Figure 2: Trophozoite *Trichomonas gallinae*. A: Independent swab check (400x) without staining. B: Swab checks using Giemsa coloring (1,000x).

Other damage that occurs due to *Trichomonas gallinae* infection was the infiltration of inflammatory cells in the mucosal and sub mucosal layers of the esophagus of the pigeons which cause thickening of the esophageal mucous layer. Histopathological images of the esophageal layer undergoing inflammatory cell infiltration can be seen in figure 3.

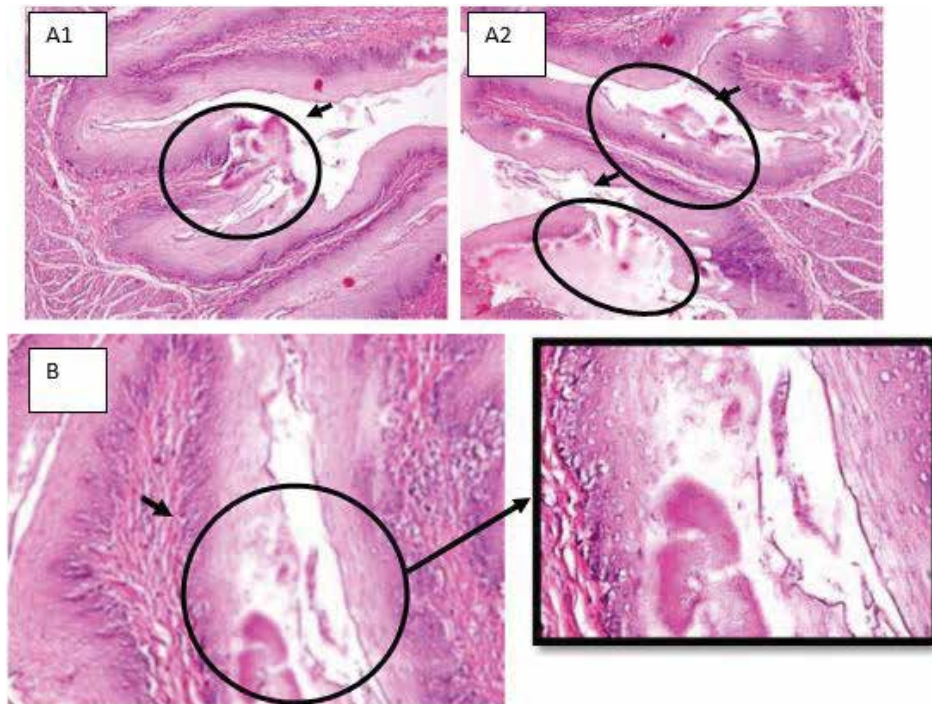


Figure 3: Histopathological features from the esophagus of naturally infected pigeons *Trichomonas gallinae* has erosion and necrosis of cells in the mucosa (H.E coloring; 100x magnification (A1 and A2) and 200x magnification (B); Nikon microscope H600L ; camera of DS Fi megapixel). (): Erosion and cell necrosis.

The results of examination and microscopic observation showed there were histopathological feature changes in the crop of pigeons with *Trichomonas gallinae* infection. These changes included cell necrosis in the mucosal layer, infiltration of inflammatory cells in the sub-mucosal layer, and there are tendons in the sub-mucosal layer. Histopathology of cell necrosis in the mucosal lining of the crop of pigeons (*Columba livia*) infected with *Trichomonas gallinae* can be seen in figure 4. Histopathology in the sub-mucosal layer experiencing inflammatory cell infiltration can be seen in figure 5. Congestion in the sub-mucosal layer can be seen in figure 6.

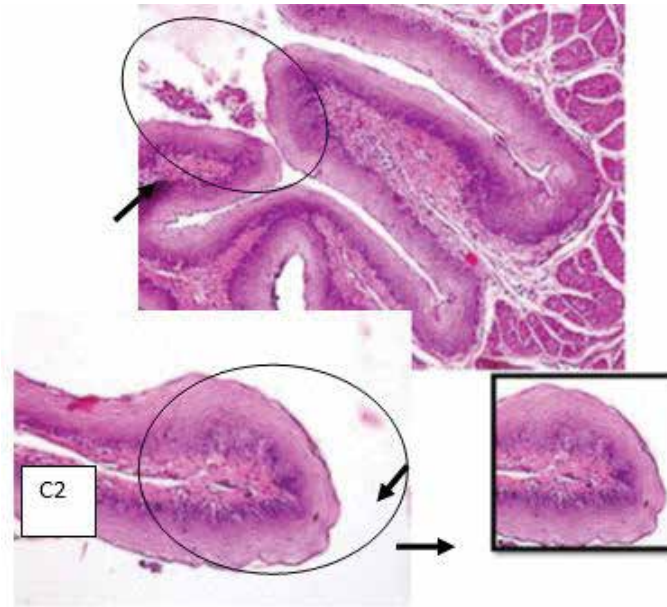


Figure 4: Histopathological picture of pigeon esophagus which has inflammatory cell infiltration due to *Trichomonas gallinae* infection (H.E staining; 100x magnification (C1) and 200x magnification (C2); Nikon H600L microscope; Camera DS Fi megapixels).

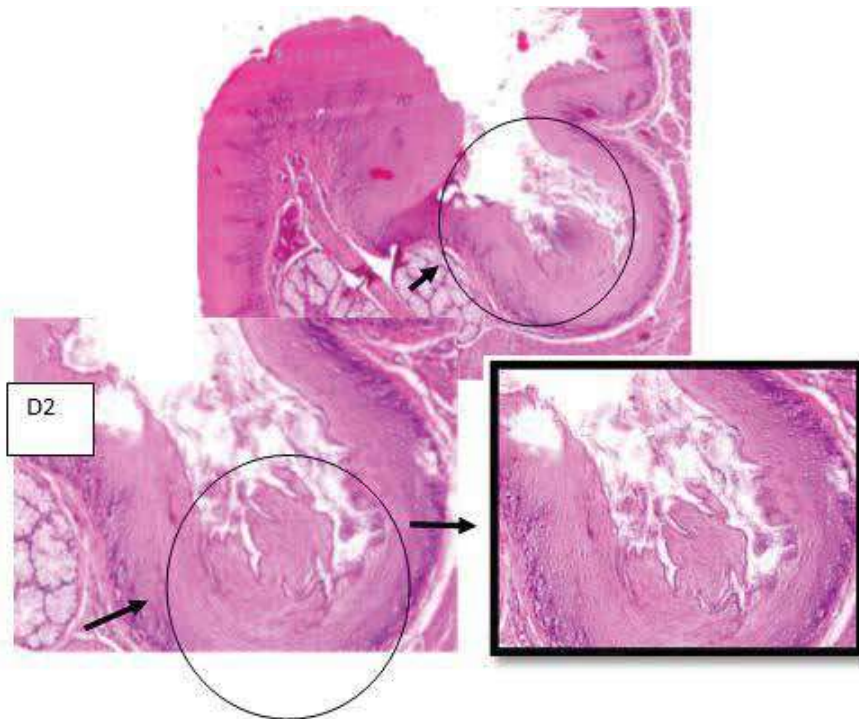


Figure 5: Histopathological features of the crop of pigeons infected with *Trichomonas gallinae*. Visible cell necrosis in the mucosal layer. (H.E coloring; 100x magnification (D1) and 200x magnification (D2); Nikon H600L microscope; camera of DS Fi megapixel).

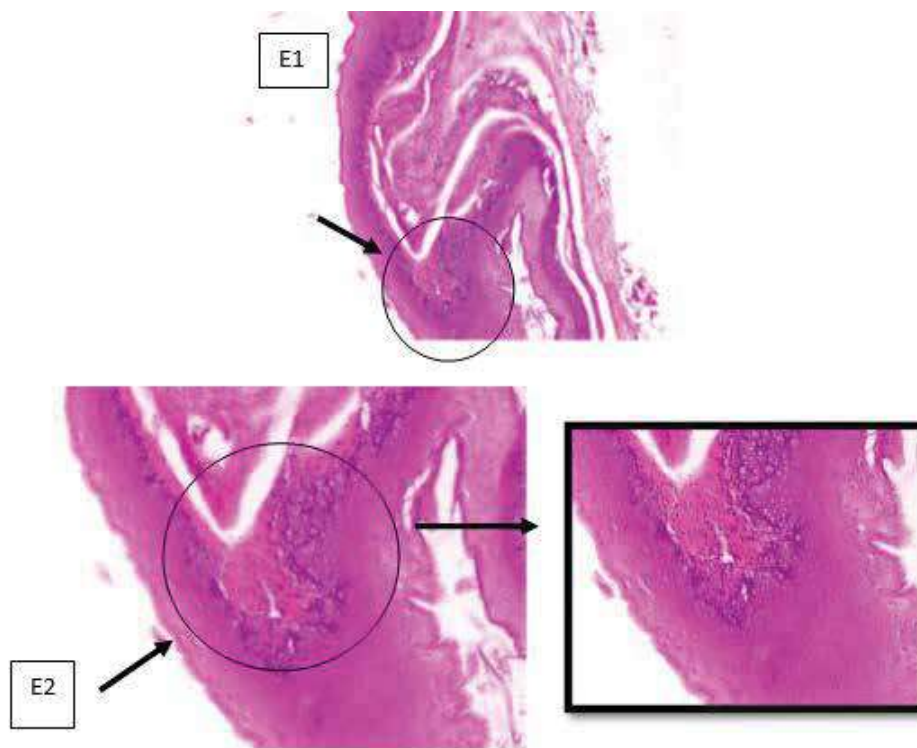


Figure 6: Histopathological features of the crop of pigeons infected with *Trichomonas gallinae*. There is an infiltration of inflammatory cells in the sub mucosal layer. (H.E coloring; 100x magnification (E1) and 200x magnification (E2); Nikon H600L microscope; camera of DS Fi megapixel).

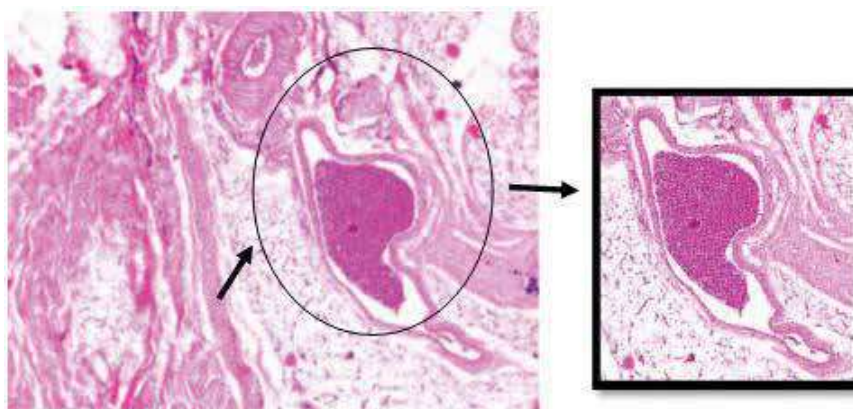


Figure 7: Visible presence of congestion in the sub-mucosal layer of the bird's nest due to *Trichomonas gallinae* infection. (H.E coloring; 100x magnification); Nikon H600L microscope; camera of DS Fi megapixel).

Discussion

Based on the results of esophagus swab examination of 20 samples of pigeons (*Columba livia*) suspected of being infected with *Trichomonas gallinae* by showing clinical symptoms of depression, weight loss, feathers stands like feeling cold and gloomy color, when standing curled up, yellow necrotic lesions were seen in the esophagus and crop, and a greenish liquid in the oral cavity.⁸ Histopathological changes were supported by the results of a research report that pigeons infected

with *Trichomonas gallinae* will experience infiltration of inflammatory cells in the mucosal layer and esophagus mucosa.¹³ In the crop of pigeons infected with *Trichomonas gallinae* showed histopathological changes of necrosis in the mucous layer and inflammatory cell infiltration in the sub layer mucosal.

Basically, *Trichomonas gallinae* infects the upper digestive tract of pigeons.¹⁴ All positive samples of *T. gallinae* detected by microscopic examination were also detected as positive by the PCR test. Most of the samples

identified negatively by microscopic examination were detected as positive *T. gallinae* by PCR testing and confirmed in sequence. Positive samples of *T. gallinae* collected will provide relevant data for studying the ecology and genetic structure of the population of *Trichomonas gallinae* and for disease prevention and control.¹⁵ The degree of change and virulence of pathological strains was low to high heterogeneity of *T. gallinae* causes trichomoniasis.¹⁶ Infiltration of the trachea and lungs is associated with a severe inflammatory response to the surrounding tissue.¹⁷

Cytopathological analysis revealed many pyriform protozoa, compatible with *Trichomonas gallinae*. Protozoa are not proven histopathologically in lesions after staining the sample with Hematoxylin and Eosin staining or Gomori Methenamine Silver (GMS).¹⁸ Histopathological changes in the esophagus of pigeons undergoing erosion and necrosis occur in the mucosal esophagus layer of pigeons. Erosion is the erosion or partial release of the mucosal epithelium. This occurs because of a traumatic reaction to *Trichomonas gallinae* infection.¹⁹ Changing histopathological of crop that undergoes cell necrosis in the mucosal layer. Changes in infiltration of inflammatory cells in the sub mucosal layer of the crop. The severity of lesions that occur as a result of *Trichomonas gallinae* infection in the upper digestive tract varies from mild mucosal inflammation to sub-mucosal inflammation.²⁰

However, the virulence of *Trichomonas gallinae* depends on other factors such as exposure to previous pathogens (protective immunity) and individual immunocompetence. Factors such as age, concurrent disease, genetic heterogeneity, geographical variation, habitat differences, abundance of means of transmission and availability of food.^{16,21} In addition, livestock should pay attention to the management of sanitary cages and feed to break the chain of distribution. Further studies regarding the degree of infection in esophageal histopathological changes and caches infected with *Trichomonas gallinae* need to be done as a step towards better diagnosis.

Conclusion

There were histopathological changes in the esophagus and crop of pigeons with infection of *Trichomonas gallinae*.

Ethical Clearance: This research has gone through ethical tests and permits from Faculty of Veterinary Universitas Airlangga

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