

# Histopathological Studies on Leucocytozoon Caulleryi Infection on Broiler in Endemic Area of Indonesia

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# Histopathological Studies on *Leucocytozoon caulleryi* Infection on Broiler in Endemic Area of Indonesia

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## ABSTRACT

**Objective:** This study aimed to observe the characteristic histopathological lesion of internal organ of broiler chicken infected by *Leucocytozoon caulleryi*. Material and Method: This study conducted in several locations in two island of Indonesia which recorded as endemic area. Clinical symptom was observed to determine the suspected animal. Subsequently, blood smear was conducted to confirm the parasitemia stage and observed the gross lesion of whole body related to pathological symptom of *leucocytozoonosis*. Every internal organ which was suspected affected, performed for histopathological preparation and examined under light microscope. The data analysis presented descriptively.

**Results:** Clinical symptom was observed from the sample population. Blood smear result confirmed that suspected chicken was due to the parasitemia of *leucocytozoonosis*. Most pathological finding showed petechiae spread in muscle tissue. Spleen, liver, lung and heart microscopic lesion showed megalozhizont. Several inflammation findings were showed in infected organ and alteration tissue order was shown in spleen.

**Conclusion:** *Leucocytozoon caulleryi* could impact the histological aspect and affect the several organs specifically with high vascularization.

**Keywords:** Chicken; Histopathology; *Leucocytozoon caulleryi*

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## INTRODUCTION

*Leucocytozoon caulleryi* one of Apicomplexa [1] species was blood protozoan which could impact on economic due to the decreased of chicken population [2]. The host of this parasite was only domestic chicken. The infection of this parasite was brought by the vector *Culicoides arakawae* midges [3] by invasion of vascular endothelium [4]. *Leucocytozoonosis* was endemic in Southeast Asian region. In Indonesia, there are positive correlation between seasonal and farm location with *leucocytozoonosis* incidence [5]. It is due to, the rise of vector population around the farm when the transition of season [6]. This phenomenon was similar with the incidence of several viruses, arbovirus and other pathogen, carried by *Culicoides sp* [7]

The histopathological and blood smear examination was needed to determine the infection. The infection of the parasite could be confirmed by the examination technique due to the different stage. It was explained that early stadium, the parasite performed schizont formation in the organ. Subsequently, the merozoite will invade the blood cell. Histopathology can define the infectious species beside the parasite stadium. *Leucocytozoon caulleryi* was the only species which found with megalozhizont inside the tissue. This study was to observe the histopathological finding due to the infection of *Leucocytozoon caulleryi* in broiler chicken.

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## MATERIALS AND METHODS

All samples (n=53) were obtained from suspected chicken with clinical symptom in two islands in Indonesia that were Kalimantan and Java. These areas consist of seven areas in Pasuruan, Lamongan, Blitar, Lumajang, Boyolali, Purwokerto and Banjarmasin city.

Suspected chicken was observed the clinical symptom subsequent, performed necropsy and observed the gross lesions of the organ. The internal organ such as heart, liver, spleen, and lung were taken and fixed in the 10% buffer formalin. The histological technique was undertaken with Hematoxylin and Eosin staining. The preparation was observed under light microscope.

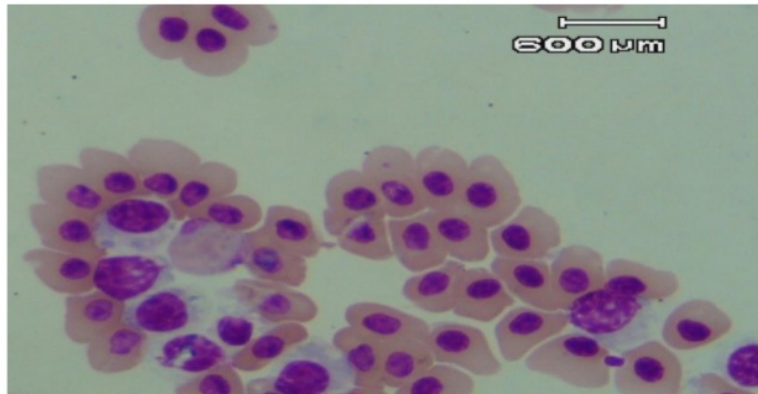
## RESULT

Chickens were obtained from 7 city in Kalimantan and Java island. In **Figure-1**, infected broiler chicken showed several clinical symptoms such as pale in the comb, and body weakness. In the cage, metallic green feces were found. Blood smear preparation showed the existence of *Leucocytozoon* parasite as seen in **Figure-2**. Parasite was seen infects the white blood cell. From all chicken that have clinical signs, *L caulleryi* was found from the sample examined **Figure-2**.

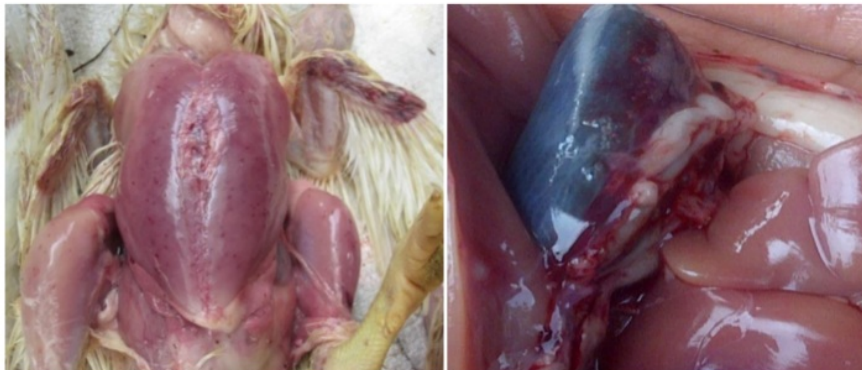


**Figure-1** Clinical finding related to *Leucocytozoonosis* infection in broiler chicken.

(A) Weakness and albescent pale in the comb



**Figure-2** *Leucocytozoon caulleryi* appearance in the blood smear of infected chicken under light microscope (HE, 1000) Gross lesions were assessed with necropsy technique. Some pathological findings were found with *Leucocytozoonosis* indication. Petechiae were found in the muscle tissue of ventral thorax and hind leg muscle as shown in **Figure-3**. Another lesion, such as hemorrhage also found in pericardial between heart and pericardium. Blood clot was found fulfill the pericardial space.



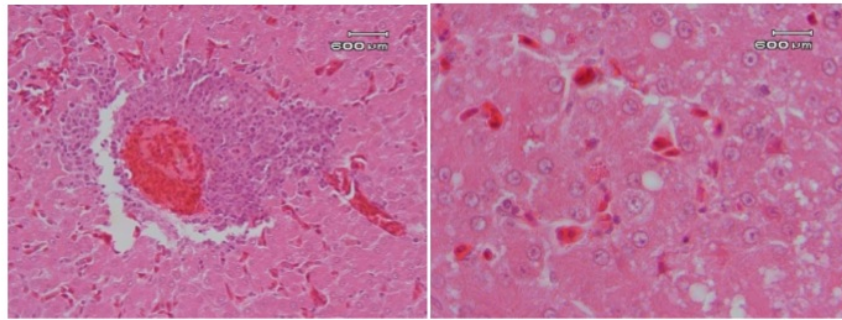
**Figure-3** Pathological anatomy of broiler chicken which suspected of being infected with *Leucocytozoonosis* infection. (A) Petechiae on the breast muscle of infected broiler. (B) Hemorrhage in pericardial space.

Histological lesion was assessed in four organs among other: heart, liver, spleen, and lung. Lesion in liver tissue was found as necrotic area in perilobular. Necrosis was followed with some congestion in the hepatic sinusoid. Hepatocyte showed lipid vacuole with HE is staining under 1000x magnification of light microscope as seen in **Figure-7**. Schizont was found in pulmonary, spleen and myocardium. In the alveolar interseptum, schizont is

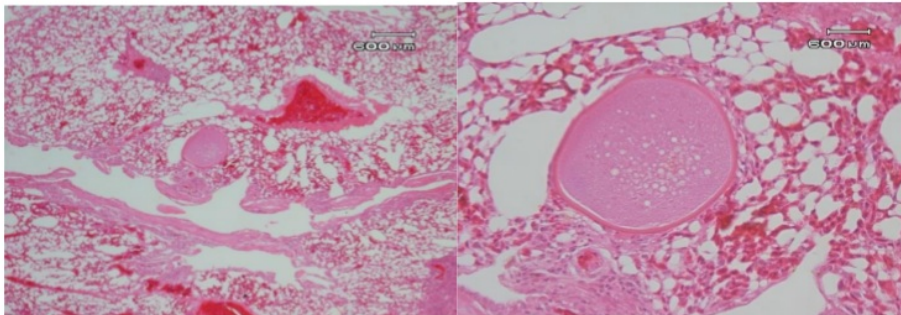
categorized as megaloschizont, a huge size of schizont in the tissue. Schizont in the liver, spleen and myocardium, as seen in **Figure-4**, **Figure-7** and **Figure-8**, are accumulated schizont, several schizont in the adjacent area inside the tissue. Congestion seen in both **Figure-5** and **Figure-6** represent the liver and pulmonary organ. Giant cell was found near the accumulated schizonts in myocardium.



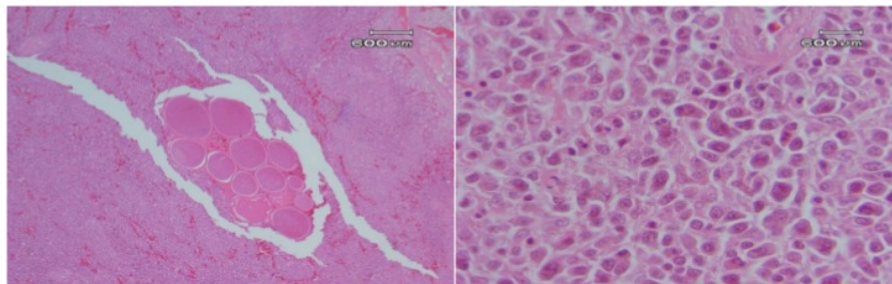
**Figure-4** Accumulation of *Leucocytozoon caulleryi* schizont in hepatic tissue. Accumulated are indicated with black arrow and seems mild congestion with white arrow.



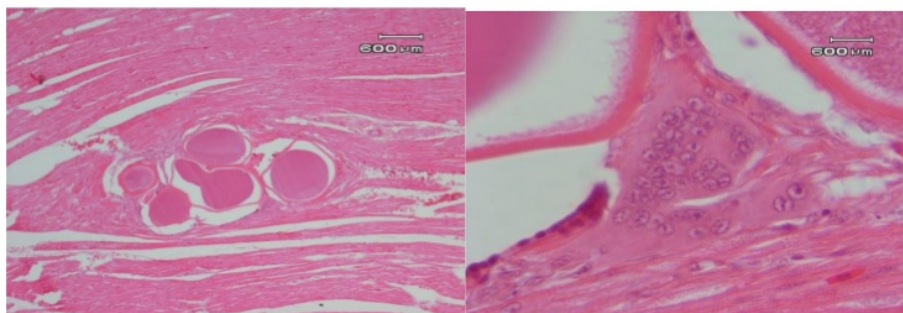
**Figure-5** Histological changes in hepatic tissue. (A) Necrotic of hepatocyte in peribiliary zone under 400x magnification of light microscope and (B) shown lipid vacuole in the hepatocyte under 1000x magnification of light microscope



**Figure-6** Histopathological finding in pulmonary tissue of infected broiler chicken. A Megalochizont of *Leucocytozoon caulleryi* in septum interalveolaris. (A) Schizont under 100x magnification and (B) under 1000x magnification of light microscope.



**Figure-7** Histopathology of infected broiler chicken's spleen. (A) Schizont and the enhancement of red blood cell is found in the interstitial of spleen tissue under 100x magnification and (B) 1000x magnification of light microscope with heterophile infiltration.



**Figure-8** Cardiac histopathology of infected broiler chicken with *Leucocytozoon caulleryi*. (A) Accumulated schizonts are found in myocardial tissue under (A) 100x magnification of light microscope and (B) 1000x magnification accompanied with Giant cell.

## DISCUSSION

Leucocytozoonosis was a strategic disease in Indonesia agriculture. It is due to its economic impact on chicken farm. It aggravated that *Leucocytozoon caulleryi* was only invade commercial chicken. An outbreak of *Leucocytozoon caulleryi* infection in farming area was related to the vector population that is *Culicoides* species [3]. *Culicoides arakawe*, vector of *Leucocytozoon caulleryi*, naturally was preferred host and seasonal dependent [7]. The dynamic of *Culicoides* midges dispersal and population are influenced by several factors including temperature, climate change, rainfall and wind speed. Wind might distribute the *Culicoides* as aerial plankton due to its ability and size to fly [8].

In Indonesia, tropical region of Southeast Asia under the equator line might contribute to the population and survival of *Culicoides* species. Thus, it influences the number and infection especially in endemic area. It was in line with the study that the succession of *Culicoides* species might impact the *Leucocytozoonosis* prevalence [9]. Endemic status in research area remain affected by seasonal change period from dried to rainy season indicating the enhancement of prevalence [5, 10]. It has correlated that the farm environments were appropriate for the habitat of *Culicoides sp.* which preferred on dirty sanitation and grassy.

The incidence of leucocytozoonosis caused several symptoms like weakness and pale [11] as seen in **Figure-2**. Leucocytozoonosis were confirmed with the metallic green of fecal found under the opened cage of flock. The symptom might misdiagnose to other disease such as malaria, chicken anemia virus, infectious bursal disease, sulfonamide intoxication, which shown the gross lesion of petechiae under the wing integument. The petechiae were present caused of the destruction of endothelial cell. It has characteristic pitted hemorrhage with cleared boundary because the leak of vascular. The destruction of erythrocyte was not severe as malaria. Although, that explained the comb pallor. The metallic green fecal is accompanied with the destruction of reticulocyte and erythrocyte [12] which makes the enhancement of excreted of blood in bile.

Evidence that strengthened the Leucocytozoonosis infection was based on the blood smear result. Blood smear result showed a positive infected leukocyte with *Leucocytozoon* protozoa as seen in **Figure-1**. This parasitemia phase clearly confirm the illness is due to the infection of blood parasite. In addition, histopathology result confirms the infection by the schizonts in the tissue. Accumulated schizonts indicate the stage of *Leucocytozoon* life cycle. Schizont was formed in the schizogony stadium. The similar result is found in studies reporting the *leucocytozoonosis* infection [3,4]. Megalochizont could be found in spleen, liver and cardiopulmonary with basophilic imaging and the eosinophilic wall as seen in **Figure-4,6,7,8** indicates the characteristic of *Leucocytozoon caulleryi* infection.

*Leucocytozoon caulleryi* has similarity to *Plasmodium* species rather than other *Leucocytozoon* species due to similar life cycle [3] and its closed phylogenicity [5]. *Leucocytozoon caulleryi* has three stadiums in life cycle named, schizogony and gametogony in host and sporogony inside the vector that is *Culicoides sp.* [3]. The schizont formation was due to the invasion of sporozoite in the endothelial cell of the chicken. The invasion of sporozoite would become first schizont subsequent, released merozoite. The second schizont was made by the

invasion of merozoite and subsequent, become microgametocyte and macrogametocyte [13]. Spleen, liver, heart and lung are the organ which has high vascularization thus it might enrich the schizonts to be survived by nutrition and oxygen supply. Spleen was the largest lymphoid organ with diverse population of immune cells [14]. This environment might support the enhancement of *Leucocytozoon* infection invading the leukocyte [15], thrombocyte [16], erythrocyte and reticulocyte [12].

The existence schizont in spleen might confirm the infection of parasite was not in early stage. Parasite of this species underwent schizogony in the endothelial and parenchymatous liver and other organ [15]. The pathological finding of spleen from microscopic lesion shows inflammation indication. Heterophile infiltration as mononuclear cell indicates the chronic inflammation. Unclear boundary between pulps distinguishes with the normal organ as seen in **Figure-7**. It might be due to the merozoites invasion to the blood cells in the spleen tissue, which a lot of blood cell. Most of young gametocyte was observed in polychrome erythrocyte and polychrome erythroblast. The second generation merozoite was infect not only the erythrocyte but also immature erythrocyte and erythroblast [12]. The merozoite also induced the inflammation process in the spleen attracting the lymphocyte, heterophyle, foreign-body giant cells, macrophage [4]

Several congestions were found in sinusoid of the liver. The leucocytozoonosis infection resulted hepatocyte necrosis and fatty degeneration with lipid vacuole remarkable as seen in **Figure-5**. Similar result found that liver tissue showed multifocal necrotic [4]. The lipid vacuole was due to the fatty liver degeneration mechanism. Whereas the decreased of hepatocyte made function of the necrotic cell was replaced to the normal cell. The liver cell was processing the lipid metabolism. The accumulation of lipid in the liver due to the decreased of the liver capacity made lipid vacuole.

In addition to liver and spleen, cardiopulmonary were impacted due to the height of vascular endothelial tissue and enriched with nutritious blood and oxygen. The host cell of *Leucocytozoon caulleryi* in the endothelial cell to multiply the merozoite induce the broken of vascularization. Subsequently, blood could get into the pericardial space as seen in **Figure-3**. It was in line, with the petechiae in muscular tissue with cleared boundary hemorrhage on the whole muscle. The invasion of merozoite into the endothelial cell was confirmed by the presence of the schizont in the heart tissue. It is found giant cell accompany near closed the schizont.

*L. caulleryi* infection can be distinguished from other diseases through organ histopathological examination. In the examined organ samples, it will usually be marked by the finding of a schizont and rupture of endothelial cells. Histopathological examination should also perform on all infected organs, not only on one organ [17].

Environmental factors have a role in the emergence of *L. caulleryi* infection. The right environment can be a good breeding place for *L.caulleryi* vectors, *Culicoides sp.* For example, there is a lot of standing water around the cage that is contaminated with chicken feces, open house system of cage, and a lot plants around the cage that are used for resting *Culicoides sp.* as a vector at daytime.

A better understanding of the ecological requirements of the immature developmental stages of potential *Culicoides* vectors and, consequently, of distribution areas

and barriers which are caused by the presence or absence of breeding habitats were very important. Such information could help predict the occurrence of biting midges in selected biotopes and improve risk assessments of culicoid-borne diseases [18].

#### CONCLUSION

Leucocytozoonosis in broiler chicken was rise in endemic area when the transition season. The histopathological finding appearance showed accumulation of megaloschizont in the tissue. The histopathological showed remarkable alteration. *Leucocytozoon caulleryi* could affect the internal organ by invasion into endothelial cell.

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