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# Identification and Prevalence of Blood Protozoa on Culling Layer Hen Sold in Wonokromo Traditional Market Surabaya City

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**Abstract**

The most common types of blood protozoa in chickens are from the phylum of Apicomplexa, including Plasmodium, Leucocytozoon and Haemoproteus. Blood protozoan diseases can cause decreased egg production, stunting and death in poultry farms. This study aims to identify and calculate the prevalence of blood protozoan infection in casted hens sold at Wonokromo Market, Surabaya City. One hundred blood samples from casted hens were taken by purposive sampling method. Blood samples were collected in Ethylene Diamine Tetra Acetate (EDTA) tubes, and then taken to the Parasitology Laboratory to make a blood smear which was then examined using a microscope with a magnification of 1000x. The results showed that the single infection was 43% positive for *Plasmodium* sp., 3% positive for *Leucocytozoon* sp. and 3% for mixed infections (*Plasmodium* sp. and *Leucocytozoon* sp.), with a total prevalence of positive samples infected with blood protozoa was 49%.

**Keywords:** laying chickens, blood protozoa, *Plasmodium* sp., *Leucocytozoon* sp.

## Introduction

Poultry is one of the livestock that can be used as a source of animal protein. Poultry could produce food in a short time and have lower price compared to beef or other large livestock meat. One of the poultry that is popular among the public is laying chickens, commonly known as layer chickens (Khalisa, 2019). The population of laying chickens in Indonesia is increasing every year, in 2019-2020 the population of laying chickens has increased by 6.5%. This increase followed by an increase in casted chickens (Directorate General of Livestock and Animal Health, 2020).

When hens production below 50%, it must be casted. Casted hens are not suitable for keeping usually sold to the market for public consumption (Soeparno, 2005). Casted hens can be a substitute for chicken is one way to meet the demand for chicken meat consumption, and as a new source of income for farmers if the selling price is high (Prasetyo *et al*., 2012).

The market is one of the places where casted hens sold and easily found by the public.

The incidence of Plasmodiosis in Indonesia varies from 1-19% (Mufasirin *et al*., 2016). The losses caused by the infestation of *Leucocytozoon* Surabaya City has many traditional markets, Wonokromo Market is one of them (Supriyanto, 2019). Wonokromo market is located in the southern area of Surabaya, it also functions as a centre for incoming goods to be redistributed to traders or other small markets. Wonokromo Market functions as the central market which is the driving force for the economy in Surabaya (Mait, 2012).

There are still many obstacles experienced by chicken farmers, one of which caused by parasitic diseases. One of the most parasitic diseases occurred in poultry, especially chickens, is caused by blood protozoa. Diseases caused by blood protozoa are one of the causes of poultry diseases that need attention, because they can be transmitted to other chickens in a short time and cause losses. The losses caused by this disease are decreased egg production, stunting and death (Solihat, 2002). One of the most important blood parasites in poultry are from the phylum of Apicomplexa including the genus *Haemoproteus*, *Leucocytozoon* and *Plasmodium* (Levine, 1994).
sp. in chicks, both broilers and laying chickens can cause clinical symptoms of 0-40% and the mortality rate reaches 7-50%; in adult chickens it

can cause clinical symptoms of 7-40% and mortality of 2-60% (Purwanto *et al*., 2009). Cases of Leucocytozoonosis in laying chickens are rare, but if Leucocytozoonosis occured then the mortality rate can reach 30% (Yesica *et al*., 2020). Research conducted by Hariani (2003), showed that 27% of the samples were positive for blood protozoa in native chickens slaughtered at traditional markets in the city of Surabaya. There are 4 markets that were sampled, namely Pucang, Wonokromo, Keputran and Kembang markets.

Malaria diseases caused by *Plasmodium* sp., *Leucocytozoon* sp. and *Haemoproteus* sp is always associated with changes in weather. During the rainy season there will be an increase in the number of vectors compared to the dry season because the rainy season is the optimum environmental condition for the development and survival of vectors that could transmit diseases. Prevention can be done by controlling the breeding of vectors (Rozendaal, 1992).

Considering the absence of data regarding blood protozoa that attack casted hens sold in the market, especially the Wonokromo Market, Surabaya City, it is necessary to conduct research on the blood of casted off laying chickens using the blood smear method, in order to obtain information about the type and prevalence of blood protozoa that can be found in casted hens sold at the Wonokromo Market, Surabaya City.

## Research Methods

This research is using Observational Type with Cross Sectional Study Design by taking samples directly at a predetermined location in the Wonokromo Market, Surabaya City. The sampling method was purposive sampling with sampling criteria based on clinical symptoms which indicates to blood protozoan infections that could be observed in the field. Examination was carried out using the blood smear method by using 100 blood samples of casted hens sold at the Wonokromo Market, Surabaya City.

The materials used in this study were 96% absolute methanol for fixation of blood smear preparations, Giemsa 10% for staining blood smear preparations, distilled water, alcohol 70% used when taking chicken blood, and immersion oil to clarify objects when observed under the microscope. Blood samples were taken through the brachial vein. These blood vessels are located in the wings of the chicken. First clean the blood collection area using a cotton swab that has been moistened with alcohol. Blood was taken by inserting a syringe in the brachial vein, after the blood came out, the blood was collected using an EDTA tube as needed. Then, label it with a description of the chicken number, date and time of collection. The EDTA tube filled with blood samples was put into a storage box. After taking the blood, the needle puncture marks on the surface of the wing skin were wiped again with a cotton swab moistened with alcohol. The collected samples were fixed and examined at the Parasitology Laboratory, Veterinary Parasitology Division, Faculty of Veterinary Medicine, Airlangga University, Surabaya.

Preparation of the smear preparation was carried out by using a pipette to collect the blood sample collected in the EDTA tube. Then drop as much as one drop on a clean object glass. Another object glass with a flat end is placed close to the drop of blood forming a 30-45$^{o}$ slope. The tip of the object glass is touched to the drop of blood so that the blood spreads to the surface of the object glass, then push the object glass so that the blood is wiped as thin as possible, then dried by aerating at room temperature. The dried blood smears were fixed with methanol solution for 3-5 minutes, then without being dried the object glass were put into 10% Giemsa solution for 30 minutes. After staining with Giemsa's solution, the blood smear was removed and washed with running water and then dried on filter paper, then examined under the microscope with 1000x magnification (Suwanti *et al*., 2017).

### Data Analysis

Data on identification and prevalence of blood protozoa in casted hens from blood examinations are presented descriptively in the form of figures and tables.

## Results and Discussions

Blood protozoa were able to be identified from 100 samples of casted hens sold at the Wonokromo Market. A single infection was found, *Leucocytozoon* sp. and *Plasmodium* sp. and mixed infections of two types of blood protozoa (*Leucocytozoon* sp. and *Plasmodium* sp.). Identification in this study is still at the genus level observed under a microscope based on morphology.

On blood examination, it was seen that *Leucocytozoon* sp infection was found in the gametocyte phase. Judging from its life cycle, it is suspected that the casted hens infected with *Leucocytozoon* sp. have been infected for a long time, so they have entered the gametocyte stage. The results of a blood smear show that the

parasite looks round or oval in shape forming a fusion with the nucleus pushed to the edge. Erythrocytes are distorted due to the larger size of the parasite and the erythrocyte nucleus is at the edge of the parasite. These characteristics are in accordance with research conducted by Arifiandani *et al*. (2019). Description of *Leucocytozoon* sp. can be seen in Figure 1.

Meanwhile, *Plasmodium* sp. infection was seen in various staging forms found in the cytoplasm around the red blood cell nucleus of casted hens. An overview of *Plasmodium* sp. can be seen in Figure 2.

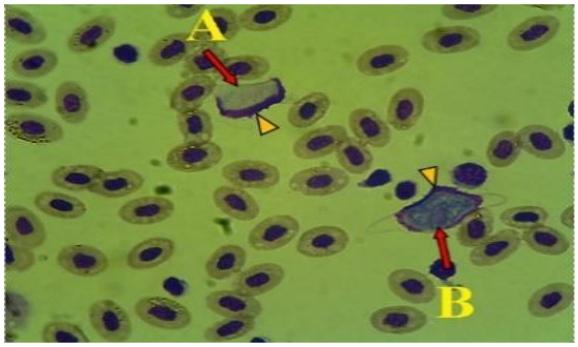
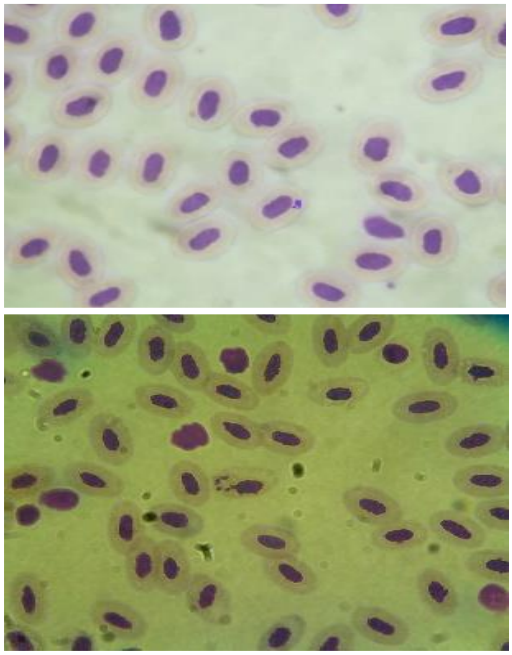
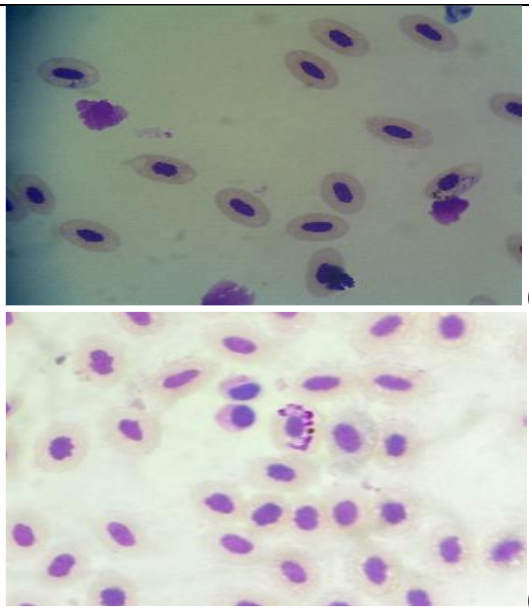



**Figure 1.** *Leucocytozoon* sp. gametocyte stage. (A) Microgametes; (B) Macrogametes. The sign (arrow) shows the gametocyte phase of *Leucocytozoon* sp. in red blood cells, the sign (triangle) shows the nucleus pushed to the edge. 1000x magnification microscope and using Giemsa staining.



(A) (B)



(C) (D)

**Figure 2.** Plasmodium sp. (A) The trophozoite stage is round or irregular in shape; (B) The old trophozoite stage (schizont) contains 5 merozoites; (C) Gametocyte stage (Microgamete); (D) Gametocyte stage (macrogamete). Signs (arrows) indicate the form of the protozoan Plasmodium sp., which is present in the cytoplasm around the nucleus of red blood cells. 1000x microscope magnification and using Giemsa staining. On blood smear examination with Giemsa staining showed *Plasmodium* sp.

Infection had entered the erythrocyte cycle, with the discovery of trophozoite, schizont and gametocyte stages around the red blood cell nucleus. The schizonts found on average only contain 3-8 merozoites, this number is still not large enough so that red blood cells do not burst. According to Soulsby (1986), red blood cells will burst when mature schizonts contain a lot of merozoites. The results showed a mixed infection of *Plasmodium* sp. and *Leucocytozoon* sp. judging from the life cycle of the two parasites, *Plasmodium* sp. takes 36-48 hours for merozoites (metacryptozoites) to enter the blood and then an erythrocytic cycle occurs for 7-10 days. Whereas, the gametocyte form of *Leucocytozoon* sp. takes about 14 days to develop in red blood cells (Mufasirin *et al*., 2016). In the different forms of infection found, it can be seen that the chickens with mixed infection were those that were first infected with *Leucocytozoon* sp. and then infected with *Plasmodium* sp. This can be due to the presence of vectors of the two parasites so that they can infect the same

chicken at different times. Blood protozoan infections caused by *Haemoproteus* sp were not found. It is suspected that *Haemoproteus* sp was not found due to the absence of *Pseudolynchia canariensis* flies as vectors of disease spreading in the livestock area. The prevalence of blood protozoa infection from 100 casted hens was 49% positive for blood protozoa infection, with each single infection 43% positive for *Plasmodium* sp and 3% positive for *Leucocytozoon* sp., and mixed infections (*Plasmodium* sp. and *Leucocytozoon* sp.) by 3%. Results of the prevalence of blood protozoa infection in casted hens can be seen in Table 1.

**Table 1**. Prevalence of blood protozoa from casted hens sold at Wonokromo Market, Surabaya City.

| Types of Blood Protozoa | | | | |
|---|---|---|---|---|
| **Single Infection** | **Amount (%)** | **Mixed Infection** | **Amount (%)** | **Total Prevalence (%)** |
| *Plasmodium* | 43 | *Plasmodium* + *Leucocytozoon* | 3 | 49 |
| *Leucocytozoon* | 3 | | | |
| Number of Samples | | | | 100 |

The results of the study show that the total prevalence obtained is 49%, this number is lower than the research conducted by Salut (2019), with the prevalence of infected blood protozoa in native chickens at the Naikoten Inpres Market, Kupang City of 56.57% and higher than the study conducted by Hariani (2003) with the prevalence of infection with blood protozoa in native chickens sold in several markets in Surabaya city of 27%. The difference in the prevalence of blood protozoa may be caused by several factors including climate, vector, age, animal immunity and how to raise chickens.

The most common blood protozoa found was *Plasmodium* sp, this is presumably due to vectors (*Culex* sp.) carrying more sporozoites from *Plasmodium* sp than vectors (*Simulium* and *Culicoides*) carrying sporozoites from *Leucocytozoon* sp. Thus, the prevalence of casted hens infected with *Plasmodium* sp was higher than *Leucocytozoon* sp. The results of research conducted by Wahyuti (2003) showed that the number of *Culicoides* vectors did not always affect the prevalence of Leucocytozoonosis, but the presence of vectors containing *Leucocytozoon* sporozoites did affect the prevalence of Leucocytozoonosis. The success of mechanical parasite transmission depends on the degree of parasitemia of the host animal, with the higher the parasite contained by the host, the greater the chance of vectors transmitting parasites to other animals (Latipah, 2009).

The results showed that the negative results of blood protozoa were more than the positive results. This result is not as expected because when reviewed from the method of collection (*purposive sampling*), which is certainly done for sampling based on clinical symptoms that lead to blood protozoa infection such as anemia, decreased appetite, very weak, depression, weight loss or thinness, the comb and wattles are pale, the balance is disturbed, the stool is green and there are petechiae or red spots on the body. So there has to be more positive blood protozoa results than the negative results.

This means that there may be other diseases other than those caused by blood protozoa, such as parasitic diseases of ectoparasites (*Monopon gallinae*, *Menacanthus stramineus*, *Dermanyssus gallinae*, etc.) which can cause anemia, decreased appetite and weight loss. In addition, it could be due to the influence of the chicken coop, which mostly uses a battery cage system which can cause the chickens to have a narrow space to move and then the transportation process is too long which makes the chickens experience stress, lose balance, etc.

Most of the preparat examinations for each microscopic whole view was only able to found one form of blood protozoa, especially *Plasmodium* sp. which means, in casted hens, which are usually old layer chickens, the possibility of blood protozoa infection in the chickens was exposed slowly so thatthe infection was still mild, and then unable to be observed by the breeder at that time. Age is a predisposing factor that can affect host immunity. Latipah (2009) said that at the cellular stage, young chickens are more easily infected than older chickens. Younger hens usually have higher number of developing parasites and longer parasitemia with higher mortality rate than older hens. Laying chickens are said to be casted off, apart from being old, they can also be casted off because their egg production is low. This decrease in egg production in laying chickens could be due to infection with blood protozoa that attack the ovaries of these hens, causing the hens to be casted off prematurely.

Based on the information from the traders, the sampling sites for the casted off laying chickens came from Blitar, Jombang, Pasuruan, Kediri, Batu and Mojokerto. This area is an enzootic area for Avian Malaria. Laying chickens that have been casted off at the Wonokromo Market after being picked up from their area of

origin, will survive in the market within 1-2 days. *Plasmodium* sp takes 36-48 hours for merozoites to enter the blood, and then erythrocytic cycle occurs for 7-10 days. While the gametocyte form of *Leucocytozoon* sp takes about 14 days to develop in red blood cells, so it is certain that the market environment has little or no effect on the occurrence of blood protozoa infection. It is suspected that the chicken was infected with a blood protozoan while on the farm.

Samples were taken during the transitional season, so there is a possibility that the cage environment is humid and there are many puddles of water which are breeding grounds for mosquitoes as vectors for spreading Avian Malaria. Cage droppings must also be considered, because it is a breeding ground for other vectors, namely flies. Chickens with depression are more susceptible to cases of Leucocytozoonosis (Widjaja, 2002). Diseases caused by *Plasmodium* sp are transmitted by *Culex* sp mosquitoes, while *Leucocytozoon* sp is transmitted by flies from the genus Simulium and Culicoides. These three types of vectors reproduce very rapidly if the environmental sanitation conditions where the hens live are very poor.

If the hens sold at the Wonokromo Market came from breeders with poor rearing patterns and in locations suitable for vector development, it is possible that the hens could be infected with *Plasmodium* sp or *Leucocytozoon* sp. and vice versa.

To reduce the incidence of blood protozoan diseases, it is necessary to take preventive measures that are carried out on an on-going basis and efficient treatment. Prevention of this disease can be done by preventing vectors by inhibiting the life cycle of the protozoa and cleaning the environment around the cage.

Treatment of Avian Malaria can use chloroquine, paludrine, pyrimethamine, sulfadiazine and clopidol (Mufasirin *et al*., 2016). In diseases caused by *Leucocytozoon* sp, the treatment that can be done is to give antibiotics that can suppress the growth of schizonts such as sulfonamides (Purwanto *et al*., 2009) and diaveridine which is a derivative of pyrimethamine, that has been proven effective when mixed into food at the level of 50 ppm (Purwanto *et al*., 2009, Mufasirin *et al*., 2016). In chickens that have been infected with blood protozoa, it can be slaughtered and the meat can be consumed or traded. All remaining pars must be destroyed by burning or burial. These preventive measures are expected to reduce the occurrence of infectious cases of blood protozoa.

## Conclusion

The types of blood protozoa found in casted hens sold at the Wonokromo Market, Surabaya City are *Plasmodium* sp and *Leucocytozoon* sp.

The prevalence of blood protozoa is 49%, with each single infection 43% positive for *Plasmodium* sp and 3% positive for *Leucocytozoon* sp. and mixed infections by 3% (*Plasmodium* sp. and *Leucocytozoon* sp.).

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