Identification and Prevalence of Blood Protozoa on Culling Layer Hen That Were 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 was 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 are 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 are sold and easily found by the public. Surabaya City has a lot of 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 is 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).
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 occurred 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, Giensa 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° 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 Arifiantri 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](image1.png)

**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.

![Figure 2](image2.png)

**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 protozoa 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 | Mixed Infection | Total Prevalence |
|------------------------|------------------|-----------------|-----------------|
| Plasmodium m            | 43 Amou nt (%)   | Plasmodium m    | 43 Amou nt (%)  |
| Leucocytozoon oo         | 3 Amou nt (%)    | Leucocytozoon oon | 3 Amou nt (%)  |
| 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 that the 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 a 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...
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