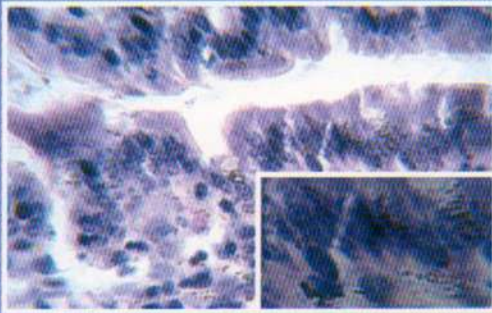
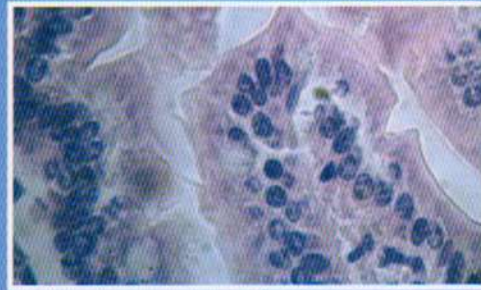
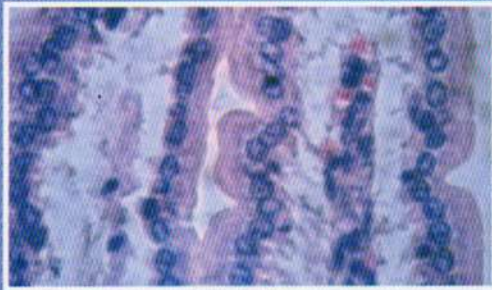


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## Protein Profile of Sporozoite of *Leucocytozoon* sp. from *Culicoides* sp.

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### Abstract

*Leucocytozoon* sp. is blood protozoa parasite that can cause leucocytozoonosis or Malaria-like. This parasite frequently infects young chicken. Transmission can be occurred by *Simulium* sp. or *Culicoides* sp. The objective of this study was to know profile of sporozoite protein of *Leucocytozoon* sp. from *Culicoides* sp. around poultry farm. This study advantage can be used as information for science development particularly parasitology. Moreover, the sporozoite protein can be developed for vaccine seed and/or diagnostic kit so that chicken leucocytozoonosis can be controlled. *Culicoides* sp. was taken from *Leucocytozoon* sp. infected poultry farm. Isolation of fly used light trap, then they were collected with aspirator. *Leucocytozoon* sp. stage in infected chicken was gamet through clearly blood smear sample. *Leucocytozoon* sp. sporozoite was isolated from *Culicoides* sp. Whole sporozoite proteins isolated and identified using SDS-PAGE. The result of the study was molecule weight of sporozoite protein of *Leucocytozoon* sp. in 37.58 kDa.

**Key words:** *Culicoides* sp., *Leucocytozoon* sp., sporozoite protein

### Introduction

Leucocytozoonosis is well known as Malaria-like, blood protozoal infection that caused by *Leucocytozoon* sp. This disease already spread to all Indonesia areas such as: Sumatra, Jawa, Bali, Sulawesi and Maluku. Particularly, Bali is endemic area. The infection attacks young as well as adult chickens. Economic loss of leucocytozoonosis is death, restricted young chicken growth and decreasing egg production. Case rate is very variation depended on vector population (*Simulium* sp. and *Culicoides* sp.). Case rate of both chicken ages can reach 40% and mortality rate is 50-60%. Chicken can be infected by biting *Simulium* sp. and *Culicoides* sp. which their salivary gland contained sporozoite.

So far, the diagnose of infection based on clinical sign, post mortem changes, the presence of gametocyte in blood smear and schizont in viseral organ. Clinical symptom of leucocytozoonosis is not specific particularly chronic infection so that frequently deceived with ILT, gumboro, fowl cholera and sulfa intoxication. The finding parasite in blood frequently is false negative due to that found in blood is gametocyte that is later stage. Thus, there is no gametocyte is not mean no leucocytozoonosis because parasite is not yet reach circulation and develop to be gametocyte. Merozoite of *Leucocytozoon* sp. and *Plasmodium* sp. is difficult distinguished. The presence

of schizont in visceral organ can be observed on post mortem. This circumstance is needed properties specific diagnosis. Suprihati *et al.* (2005) has been done protein isolation of *Leucocytozoon* sp. schizont but that protein is not developed yet as diagnostic tool. Schizont is formed one week after infection by sporozoite so that infection course has done. The death of chicken is occurred due to blood diarrhea, severe anemia and visceral organ damage such as liver, brain, lung, kidney dan heart by broken schizont. Infective stage of *Leucocytozoon* sp. is sporozoite so that the first infection agent well known by immune system is sporozoite. Up to now in Indonesia, the development of diagnostic kit for leucocytozoonosis from sporozoite of *Leucocytozoon* sp. is not reported yet.

Up to now diseases prevention are performed by suppressing vector population and limitation of vector contact. Generally, depopulation are done by spraying insecticide frequently growing up resistance. The contact limitation with vector is difficult to avoid because the size of vector is small and the distance of fly is far so that possible the vector enter livestock barn. This condition is needed controlling through vaccination. Thus, the use of sporozoite protein as vaccine materials hoped *Leucocytozoon* sp. infected chicken from salivary gland of vector; sporozoite will not develop and result in infection.



In order to develop vaccine materials and/or diagnostic kit materials are needed identification of sporozoite. This study wants to know protein profile of sporozoite of *Leucocytozoon* sp. The result of this study was hoped a protein product that can be developed seed vaccine and/or diagnostic kit for controlling leucocytozoonosis.

### Materials and Methods

The study was initiated May until August 2009 at poultry farm on Kediri regency and Department of Veterinary Parasitology Faculty of Veterinary Medicine, Surabaya.

Samples were chicken blood suspected *Leucocytozoon* sp infection in poultry farm on Kediri regency and trapped *Culicoides* sp. around *Leucocytozoon* sp. infected chicken barn. Data of the result of fractionation were presented by descriptive.

#### Isolation of *Leucocytozoon* sp.

Isolation was done by blood smear examination of several chickens of *Leucocytozoon* sp. infected poultry farm. *Leucocytozoon* sp. infected chicken was presented gametocyte *Leucocytozoon* sp. in erythrocyte as well as leukocyte.

#### Catch of *Culicoides* sp.

*Culicoides* sp. was taken from around barn of *Leucocytozoon* sp. infected poultry farm based on blood smear examination. The catch of *Culicoides* sp. used light trap. The light trap installed around barn. The installing light trap was performed to begin 05.00 to 08.00 p.m. Caught *Culicoides* sp. were sucked with aspirator then entered smooth gauze plastic. The *Culicoides* sp. was identified in laboratory Entomology and Protozoology Laboratory, Department of Veterinary Parasitology of Faculty of Veterinary Medicine, Airlangga University.

#### Isolation of *Leucocytozoon* sporozoite from *Culicoides* (Akaki and Dvorak, 2005)

Catched *Culicoides* sp. was anaesthetized using chloroform. The fly entered 0.5 ml of eppendorf tube. The base of tube perforated with 20 gauge of needle, diameter of hole about 900  $\mu$ m. One hundred  $\mu$ l of glass wool entered tube and added 200  $\mu$ l of medium of M 199 so that the surface of medium was the surface of glass wool, then tube entered in tube. Tube 0.5 ml entered in tube 1.5 ml, centrifused 16 000 g for 3 minutes. Sporozoite would be collected as pellet in the base of eppendorf 1.5 ml. Then sporozoite was observed by microscope of 400 - 1000x.

#### Protein isolation and identification of sporozoite from *Leucocytozoon* sp.

Sporozoite was washed 3 times using suspension of PBS and centrifused 10.000 rpm for 10 minutes, respectively. Sporozoite resuspended with 2 ml of PBS and counted with haemocytometer.

Sporozoite resuspended with PBS so that concentration per ml was  $1 \times 10^8$  sporozoites and added protease inhibitor. Sporozoite sonicated by frequency of 4 x 1 minutes. The result of sonication entered dialysing tube and dialysed overnight at 4°C. Concentration of protein measured with spectrophotometer.

Identification was performed using fractionation and determination of molecule weight of whole sporozoite protein with 15% of SDS-PAGE. Protein entered into eppendorf tube and added buffer of sample then boiled in boiling water for 2 minutes. Sample and standard protein (marker) entered column of stacking gel and running until all sample to reach the base of gel. Gel stained with commasie blue. Molecule weight counted with regression coefficient of molecule weight standard and migration length per protein fraction.

### Results and Discussion

From 5 poultry farms observed in Kediri regency were found one of *Leucocytozoon* sp. infected poultry farm. Chicken blood was taken from Leucocytozoonosis showed clinical sign chicken (i.e. weakness, pale and blood spots in pectoral muscle). Then, blood smear examination was shown gamet stage (Fig. 1).

Transmission of *Leucocytozoon* sp. is occurred by vector biting. Chicken infected due to biting of blood sucking fly (*Simulium* and/or *Culicoides*) which their salivary gland contain sporozoite. According to Sukardono (1986) in Indonesia, of those flies, *Culicoides* sp. has the role as leucocytozoonosis vector on chicken and several *Culicoides* species has been identified, but among those species that the most suspected as leucocytozoonosis vector on chicken was *C. arakawae* and *C. guttifer* (Sukardono, 1987). Wahyuti (2003) reported that around poultry farm in Jombang area that positive leucocytozoon infected was at least there was six species of *Culicoides* sp.

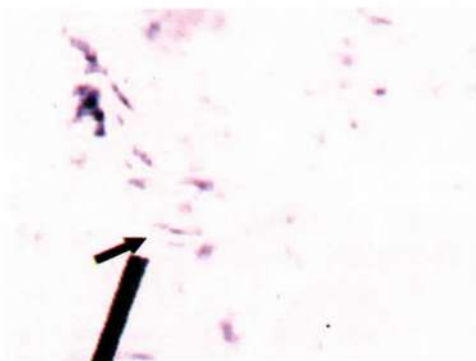
This study was not distinguish *Culicoides* sp. Figure 2 showed one of fly catch. All caught flies were anaesthetized for isolating sporozoite. dianastesi semua untuk diisolasi sporozoitnya. Sporozoite of the isolating result was shown at Fig. 3.



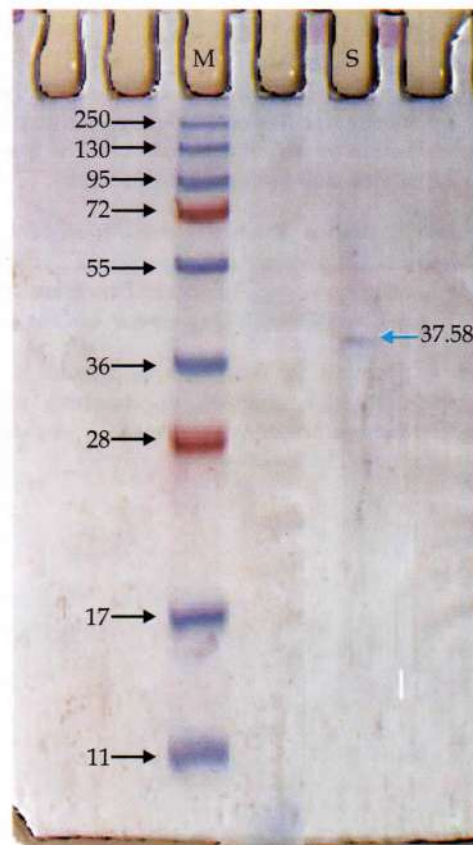
**Figure 1.** Blood smear of *Leucocytozoon* sp. infected chicken. Arrow (→) showed gametocyte of *Leucocytozoon* sp., (magnification of 1000x).



**Figure 2.** *Culicoides* sp. from around barn of poultry farm



**Figure 3.** Sporozoite of isolation from *Culicoides* sp. (magnification of 1000x)



**Figure 4.** Result of analyzed of sporozoite protein of *Leucocytozoon* sp. M, Marker Protein; S, Sporozoite protein.



The result of electrophoresis using SDS was only found one band of protein of 37.58 kDa of molecule weight. Up to now, there is no report about profile of sporozoite protein isolated from *Culicoides* sp. Several studies of researchers are *Leucocytozoon* sp. protein from schizont. Suprihati *et al.* (2005) reported *Leucocytozoon* sp. schizont protein of molecule weight of 68.2, 55.2, 49.7 and 44.7 kDa, respectively. Isobe *et al.* (1998; 2000) reported that there was the differences protein molecule weight that reacted with antibody from natural infected chicken serum and immunized chicken. Serum of *L. caulleryi* infected chicken showed band of antibody reaction with protein on molecule weight of 33, 44, 58, 79, 94, and 141 kDa, respectively. Whereas immunized chicken with schizont of *L. caulleryi* showed band of reaction with protein on molecule weight of 36, 58, 71, 81, 97, 112, and 123 kDa.

### Conclusion

*Leucocytozoon* sporozoite protein that isolated from *Culicoides* had 37.58 kDa of molecule weight.

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