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Leukocyte Differential Study in Wild Hawksbill Turtles (Eretmochelys imbricata)

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Abstract

Wild hawksbill turtles (*Eretmochelys imbricata*) can be affected by various diseases which generally affect the respiratory system, digestive system, metabolic system, bones, skin and reproductive system. One way to help diagnose disease is through blood tests, such as calculating leukocyte differentiation. Leukocytes are one of the blood cells which have the main function of protecting the body from pathogen attacks. Leukocytes consist of neutrophils, eosinophils, basophils, monocytes and lymphocytes. The aim of this research is to provide information about leukocyte levels in wild hawksbill turtles (*Eretmochelys imbricata*) on Liukang Loe Island, Bulukumba Regency. The method used in sampling turtles was random sampling. Blood samples are taken from the supravertebral sinus (dorsal cervical vein) in the neck using a syringe. Then preparations were made by adding methanol and then staining using Giemsa solution. The preparations were observed under a microscope with 40x magnification. Then each type of leukocyte was counted until the number was 100 leukocyte cells. The observation results obtained in one hawksbill turtle were 78 lymphocytes, 22 monocytes, 195 neutrophils, 3 eosinophils and 2 basophils. In second hawksbill turtles, the results were 50 lymphocytes, 56 monocytes, 64 neutrophils, 2 eosinophils and there are no basophils. The differentiation results obtained in one hawksbill turtle were lymphocytes with a percentage of 26%, monocytes 7.3%, neutrophils 65%, eosinophils 1% and basophils with a percentage of 0.6%. In hawksbill turtles the two percentages obtained were 16.6% lymphocytes, 18.6% monocytes, 64% neutrophils, 0.65% eosinophils and no basophils.

Keywords: *Eretmochelys imbricata, Leukocytes, Wild hawksbill turtles, Liukang Loe Island*

Introduction

The largest archipelagic country in the world is Indonesia, because it has more than 17,500 islands and 360 million hectares of sea area. The location of Indonesia's marine areas is very suitable for the growth of coral reefs, seaweed and biodiversity including sea turtles (Limpus and Mclachian, 1996).

One of the protected animals in the world is the turtle. Turtles are protected because their population is threatened with extinction. Turtles are able to move very long distances

throughout the Indian Ocean, Pacific Ocean and Southeast Asia. Six of the seven types of turtles in the world are found in Indonesia. The types of turtles found in Indonesia are the Green Turtle (*Chelonia mydas*), Hawksbill Turtle (*Eretmochelys imbricata*), Olive Ridley Turtle (*Lepidochelys olivacea*), Leatherback Turtle (*Dermochelys coriacea*), Flatback Turtle (*Natator depressus*) and Loggerhead Turtle (*Caretta caretta*) (Ario et al., 2016).

Hawksbill turtles are animals belonging to the Chelonidae family (*Eretmochelys imbricata*) along with five other types of turtles. Hawksbill turtles have special physical characteristics in the form of a beak that resembles a bird and carapace scales that are arranged in imbricates (overlapping). Hawksbill turtles are distributed in almost all tropical and sub-tropical waters of the world, including Indonesian waters (Prakoso et al., 2019). One of the locations with the largest population of hawksbill turtles in South Sulawesi is Bulukumba Regency, especially on Liukang Loe Island.

Liukang Loe Island is one of the tourism areas in Bulukumba Regency and is one of the tourist destination areas (DTW) in Bulukumba Regency which has the potential to contribute to increasing local revenue (PAD). Liukang Loe Island is very unique with unique cultural characteristics of the local community and physically the island area is surrounded by white sand, coral reefs and turtles which can support marine tourism activities such as beach tourism activities (recreation/relaxation), snorkeling and diving (diving) (Juhannis, 2015). This marine tourism activity basically contributes to economic growth and the level of welfare of the people there. However, on the other hand, these activities also have a negative impact on the sustainability of coastal resources, especially damage to coral reefs and a decline in marine biota populations, especially hawksbill turtles (Rajab et al., 2013).

The turtle population on Lukang Loe Island is also experiencing a decline due to various factors such as environmental pollution due to human activities, as well as illegal catching or taking of eggs and adult turtles as well as disease or health problems (Adnyana and Hitipeuw, 2009). Among the factors causing population decline, disease or health problems are still very poorly understood. One way to help diagnose a disease is to carry out a blood test procedure (Colville and Bessert, 2013). Blood tests are carried out to confirm the diagnosis of a disease. The description of blood parameters (hematological) is a supporting aspect in determining the health status of animals (Utami et al. 2013). Diseases that commonly attack reptiles are disorders of the respiratory system, digestive system, metabolic system, bones, skin and reproductive system (Nurkarimah, 2019).

Based on the description above, this research was conducted to observe differences in white blood image parameters in turtles. The blood picture parameters that can be studied are total leukocytes, lymphocytes, monocytes and neutrophils. Leukocyte differentiation in turtles is carried out so that in the future it can be used as a basic reference in diagnosing health status in hawksbill turtles.

Meterials and Methods

This research is descriptive research with exploratory activities. Data collection was carried out by direct observation in the field by taking blood samples from hawksbill turtles on Liukang Loe Island, Bulukumba Regency.The method used in sampling is random sampling. The number of hawksbill turtles whose blood was collected was 2 with a blood volume of 3 ml each. The sample used in this research was hawksbill turtle blood. The research tools used in this research were a 1 cc spout, a 3 cc spout, light microscope, object glass, cover glass, cellphone camera, roll meter, ice box cooler and 4 sets of snorkeling equipment.
Turtles will be caught using the long-term mark recapture method (Lanyon et al 1989). Turtles are caught with bare hands while snorkeling during the day at two dive sites set at

a depth not exceeding 10m. The turtles were transferred to boats that had been modified to reduce stress levels in the animals. Blood collection from hawksbill turtles is carried out in the supravertebral sinus (dorsal cervical vein) in the neck. The blood collection procedure is carried out by wiping the dorsal part of the neck using sterile cotton soaked in alcohol. Then the neck is straightened and the head is held, so that the sinus is located just lateral to the cervical vertebrae. The syringe needle is inserted at a 30° angle into the neck at a location one third of the distance from the base of the head to the carapace and one third of the distance from the dorsal midline to the lateral edge of the neck with the needle hole facing upwards (Campbell, 2015). After the needle is inserted (marked by the presence of blood at the tip of the syringe), aspiration is carried out to collect blood. After the blood collection procedure is complete, the hawksbill turtle is released back into its habitat.

Making blood smear preparations is done by placing a drop of blood on an object glass. The second object glass is placed at a 45° angle on top of the first object glass, then moved backwards to touch the blood so that the blood spreads. The second object glass is then shifted in the opposite direction to form a thin layer of blood. Blood smear preparations were allowed to dry in air. After that, the fixation process was continued, by soaking the preparations in methanol for 5 minutes, then drying. The preparations were then given Giemsa solution for 30 minutes, after which they were washed and dried. Next, the preparations were observed under a microscope with a magnification of 40x, and each type of leukocyte was counted until the number of 100 leukocyte cells was reached.

$$Jumlah\ Leukosit\ Total\ (\%) = \frac{komponen\ sel\ leukosit}{100} \times 100\%$$

Figure 1. Leukocyte calculation formula (Widyaningrum et al., 2017).

Data analysis

Data were analyzed descriptively. The parameters observed were leukocyte differentiation which included the percentage of neutrophils, eosinophils, basophils, lymphocytes and monocytes.

Results and Discussion

This research was conducted to determine leukocyte differentiation in wild hawksbill turtles (*Eretmochelys imbricate*) on Liukang Loe Island, Bulukumba Regency. This research is descriptive research with exploratory activities. A total of 2 wild hawksbill turtle blood samples were collected by random sampling. Sample examination was carried out at the Histopathology Laboratory of the Veterinary Education Clinic, Hasanuddin University.

In general, the diversity of leukocyte types in reptiles is classified into two groups, namely granulocytes consisting of neutrophils, basophils and eosinophils, and agranulocytes, namely lymphocytes and monocytes (Rovira 2010). The existence of leukocytes is very important because each type has a role and describes health status. Table 1 presents a comparison of leukocyte differentiation in wild hawksbill turtles found in this study.

Table 1. Comparison of leukocyte differentiation in wild hawksbill turtles

Turtle Code	Lim	Mon	Neu	Eos	Bass
P1	26%	7.3%	65%	1%	0.6%
P2	16.6%	18.6%	64%	0.6%	-

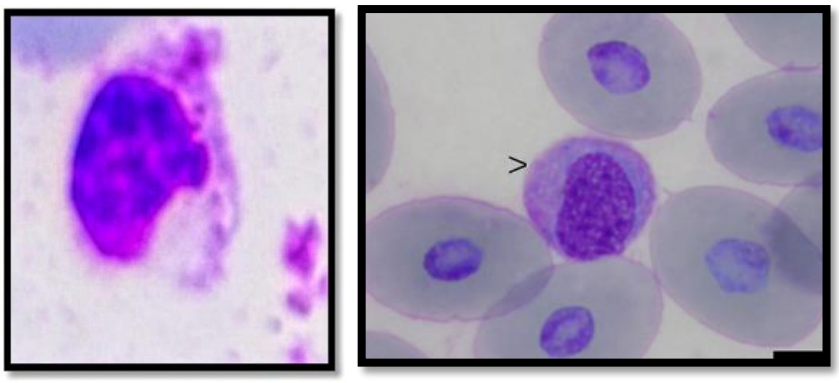
Type: P (Turtle), Lim (Lymphocyte), Mon (Monocyte), Neu (Neutrophil), Eos (Eosinfosil), Bas (Basophil)

Leukocytes are the most active unit of the body's defense system and are in the blood circulation in various ways. The number of leukocytes is less than that of angry blood cells. The main function of leukocytes is to destroy infectious and toxic materials through the process of phagocytosis by forming antibodies. Leukocytes are a blood component that functions as a non-specific defense (Erika, 2008).

1. Lymphocyte Percentage

The observation results obtained were that the percentage of lymphocytes in turtle 1 was 26% and turtle 2 was 16.6%. The percentage obtained is still within the normal range. According to Coppo et al (2005), the percentage of lymphocytes is usually 16.3% to 39.8%. Lymphocytes have a very important role in the immune response and producing antibodies. In normal lymphocytes the cytoplasm appears homogeneous, generally does not have vacuoles, granules, and is slightly basophilic (pale blue) (Dzaky, 2018).

Lymphocytes function as producers of antibodies to deal with disease disorders. There is a decrease in the percentage of lymphocytes in the circulation during an infection, thought to be due to the disruption of the activity of lymphocytes in producing antibodies, then in this condition the lymphocytes are assisted by monocytes and neutrophils whose numbers will increase in the circulation, in the blood circulation of higher animals which play an important role as Cellular immunity is important for protecting the body from intracellular bacteria and viruses (Rustikawati, 2012).



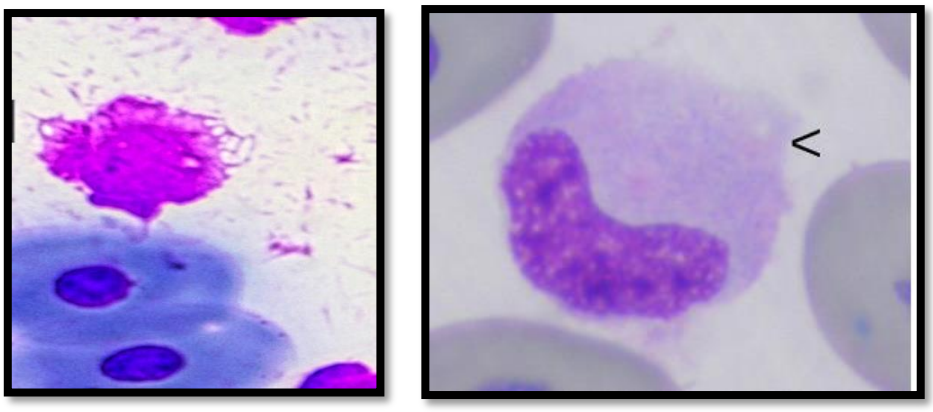
(a) (b)

Figure 2: Overview of turtle lymphocytes (a) Lymphocytes observed, (b) Lymphocytes observed by Casal and Oris (2007).

These reptile lymphocytes are also divided into two, namely B cells and T cells. T cells are processed in the thymus before going to peripheral lymphoid tissue. T cells are responsible for immunity (no production of antibodies) and for activating B cells. Most of the lymphocytes in the peripheral blood are T cells. Inactive B cells travel through the lymph nodes, spleen and lymphoid structures but are rarely present in the peripheral blood. B cells are responsible for humoral immunity (antibody production). Each B cell is programmed to produce only one specific type of antibody (Colville and Bassert, 2016).

2. Monocyte Percentage

The observation results showed that the percentage of monocytes in turtle 1 was 7.3% and turtle 2 was 18.6%.According to Dzaky (2018), the percentage of monocytes is usually between 0% and 10%. Turtle 1 had a monocyte percentage within normal limits while turtle 2 had an increase. An increase in the percentage of monocytes in the leukocyte count indicates inflammation. A high percentage of monocytes above the normal value range indicates a leukocyte response to foreign objects in the body (Giyartika and Keman, 2020).



(a) (b)

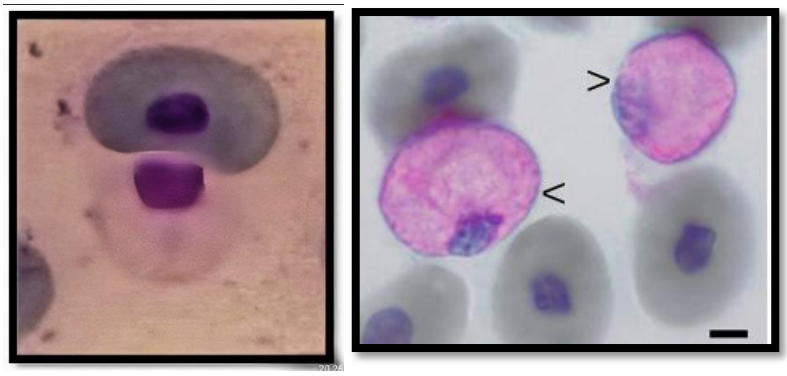
Figure 3: Overview of turtle monocytes (a) Monocytes observed, (b) Monocytes observed by Casal and Oris (2007)

Monocytes are the largest circulating leukocytes. Monocytes will be attracted if injury occurs. Macrophages have a major role and regulation of inflammation and immune responses (Fails and Magee, 2018). Monocytes change into macrophages when they are seen entering the tissue and are often found in areas of inflammation or infection. That when an infection occurs by a foreign body, monocytes will move quickly from the blood vessels to the infected area or in other words diapedesis to carry out phagocytosis. Monocytes have the ability to penetrate capillary walls, then enter tissue and differentiate into macrophages (Affandi and Tang, 2002).

Monocytosis is an increase in the number of monocytes in the blood. Meanwhile, monocytopenia is the opposite of monocytosis, namely a decrease in the number of monocytes in the blood. Monocytes participate in inflammation. Monocytes do not spend much time in the bone marrow before entering the tissue, when monocytes enter the tissue the monocytes change their name to macrophages (Colville and Bassert, 2016).

3. Neutrophil Percentage

The observation results showed that the percentage of neutrophils in turtle 1 was 65% and turtle 2 was 64%. The percentage obtained is still within the normal range. According to Coppo et al (2005), the percentage of neutrophils usually ranges from 40% to 86.1%.Neutrophils capture and destroy invading microorganisms, through phagocytosis and intracellular degradation, release of granules, and formation of neutrophil extracellular traps upon detection of pathogens. Neutrophils also participate as mediators of inflammation (Rosales, 2018).



(a) (b)

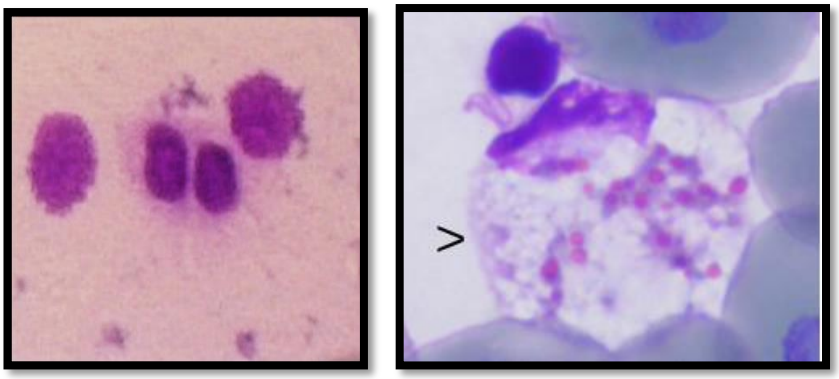
Figure 4: Image of turtle neutrophils (a) Observed neutrophils, (b) Observed neutrophils by Casal and Oris (2007)

Neutrophils are the most abundant leukocytes, making up approximately 90% of all granulocytes. Neutrophils are able to move through the endothelial lining of blood vessels into the surrounding tissue and engulf invading bacteria and cell debris by phagocytosis, so they help fight disease. Neutrophilia or an increase in the number of neutrophils indicates an infectious process. While neutropenia or lack of white blood cells is a characteristic of certain viral infections (Aspinall and Melanie, 2015).

Neutrophils are the first leukocytes in the defense system against infection. After injury, neutrophils quickly accumulate in the fluid in the injured area. Neutrophils will attack bacteria and destroy them. In the process of destruction, neutrophils involve the action of enzymes in intracellular granules and enzymes. During phagocytosis, neutrophils can release enzymes that contribute to local inflammation. Increases and decreases in the percentage of neutrophils can be caused by the time neutrophils are in blood circulation. Neutrophils are a homogeneous population of terminally differentiated cells with unique functions (Fails and Magee, 2018).

4. Eosinophil Percentage

The observation results showed that the percentage of eosinophils in turtle 1 was 1% and turtle 2 was 0.6%. The eosinophil percentage was still within the normal range. According to Frye (1991) the percentage of eosinophils in reptiles is around 0-1% in the blood circulation.The leukocyte component associated with parasitic infections is eosinophils, so the appearance and increase of eosinophils indicates the number of parasites (Mahasri et al., 2011).



(a) (b)

Figure 5: Image of turtle eosinophils (a) Observed eosinophils, (b) Observed eosinophils by Casal and Oris (2007).

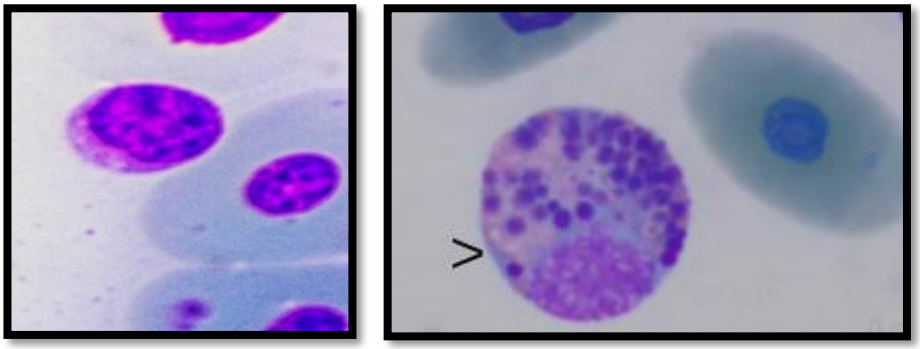
Eosinophils have two special functions, namely, the first is that they are able to attack and destroy worm larvae (parasites), while the second function is that the enzymes produced by eosinophils are able to neutralize inflammatory factors released by mast cells and basophils in the process of type 1 hypersensitivity. Eosinophils play an active role in regulating the allergic process. acute and inflammatory, regulate parasite infestation, and phagocytose bacteria, antigen-antibody complexes, mycoplasma, and yeast. Eosinophil cells also contain histaminase which activates histamine and releases serotonin from certain cells, and also releases zinc which blocks platelet aggregation and macrophage migration (Lokapirnasari and Yulianto, 2014).

The main function of eosinophils is to neutralize the presence of toxic substances, so that their presence in large numbers in certain places is related to the presence of antigen-antibody reactions and in certain places they penetrate foreign substances in the body (Lokapirnasari and Yulianto, 2014).

Eosinophils are produced during parasitic infections and during allergic reactions. During an allergic reaction, mast cells and basophils release eosinophil chemotactic factors so that the eosinophils migrate towards the inflamed tissue and the increase in eosinophils is caused by several conditions such as hypersensitivity, for example due to parasites and allergies, the healing stage of acute infections, tumors and insufficiency of adrenal cortex products. On the other hand, increased eosinophils can also be caused by inflammatory diseases, mast cell disorders, hypoadrenocorticism (Lokapirnasari and Yulianto, 2014).

5. Basophil Percentage

The observation results obtained showed that the percentage of basophils in turtle 1 was 0.6% and in turtle 2 no basophils were found. The percentage of basophils from these observations is in the normal range. According to Hampel et al (2009), the normal basophil percentage in turtles ranges from 0-4%.



(a) (b)

Figure 6: Description of sea turtle basophils (a) Observed basophils, (b) Observed basophils by Casal and Oris (2007)

Basophils have the same function as basophils in mammals. This is why the number of basophils will vary between species, which is influenced by physiological conditions such as parasitic infections.Basophils play a role in parasitic infections and allergic responses, which are associated with acute illnesses. Basophilia is an increase in the number of basophils in the blood. The causes of an increase in the number of basophils are food, drugs and parasite bites. Basopenia is a decrease in the number of basophils in the blood. Causes of decreased basophil counts include urticaria and anphylaxis (Weiss and Wardrop, 2010).

Basophils play an important role in the body's immune response, which begins upon contact with allergy-causing substances by producing chemical mediators such as histamine which then attract other immune cells. Basophil cells contain heparin, histamine, hyaluronic acid, chondroitin sulfate, serotonin, and several chemotactic factors. Basophils act as mediators for inflammation and allergic activity, have immunoglobulinE (IgE) and immunoglobulin-G (IgG) receptors which cause degranulation and evoke hypersensitive reactions with vasoactive secretions (Lokapirnasari and Yulianto, 2014).

Conclusion

The differentiation results obtained in the two turtles were in 1 turtle lymphocytes with a percentage of 26%, monocytes 7.3%, neutrophils 65%, eosinophils 1% and basophils 0.6%. And in turtles there are 2 lymphocytes with a percentage of 16.6%, monocytes 18.6%,

neutrophils 64%, eosinophils 0.6% and there are no basophils.

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