Devagiri Journal of Science 8(1),20-27

www.devagirijournals.com
ISSN 2454-2091

Comparative Analysis of Blood values in Selected Diseased and Recovered Dogs

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Received: 12.08.2021

Revised and Accepted: 15.9.2022

Key Words: Comparative analysis, CBC analyzer, bacterial pneumonia, bacterialmyositis, lymphopenia

Abstract

Early diagnosis of disease plays an important role in management of various diseases. Any diagnostic aid which helps the clinician to arrive in an appropriate diagnosis in the field level is more acceptable than the sophisticated equipments. In this study we selected five animals infected with different etiological agents like bacteria, virus and parasite. Obtained complete blood count by CBC analyzer along with manual differential leukocyte count. Compare the results and the blood profile obtained by CBC analyzer was almost in conjunction with the result received by manual counting method. Changes in the count of different WBC's during various infections were analyzed and concluded that an increase in neutrophil were observed in bacterial infections like bacterial pneumonia and bacterialmyositis and lymphopenia were observed in viral infections like canine parvoviral enteritis and canine distemper. Parasitic infection was associated with an increase in eosinophils. In this study, mere blood smear examination helped us to arrive in a diagnosis. So, it can also be concluded that blood smear examination can be used as a quick diagnostic aid in less equipped laboratories.

1. Introduction

Diagnosis plays significant role in early intervention of various diseases in animals. Diagnostic testing is used to assess internal elements of pet for early detection of disease, or to confirm suspected diagnoses that have been based on symptoms that the animal has been present with. There are different diagnostic aids available but most of them require sophisticated equipment's. Despite the widespread availability of automated analyzers, the value of basic blood smear should not be forgotten and should be incorporated into routine blood testing in clinical practice. Blood is one of the important body fluids to assess the health status of animal. The evaluation of blood parameter is the first step in diagnosis of many diseases (Ariyibi *et al*.,2002). Hematologicaltests are routinely done to determine the general level of health in animals and to distinguishing them from diseased ones. Interpretation of blood smear is commonly used to provide rapid laboratory evaluation of animals in veterinary emergency practice (Barger, 2003).

The differential Leukocyte Count (DLC) sometimes referred to as the White Blood Cell differential (WBC differential), or simple differential, remains one of the most frequently performed test in a hematology laboratory. Differential cell counts typically are enumerations of the different White Blood Cells (WBC)circulating in the blood. It has become widely accepted and used by clinicians and is generally considered to yield clinically useful information in health and disease. (Dear, 2014). The WBC differential count is often used to

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monitor a diseased animal's progress and /or response to treatment. The differential leukocyte count can be of two type; Manual differential leukocyte count and automated differential leukocyte count. The manual differential leukocyte count is performed by having a person trained in peripheral blood morphology review the stained blood smear and manually count 100 white cells. The latter is performed by a blood sample which is loaded onto an analyzer, which samples a small volume of blood and measures various properties of white blood cells to produce a differential count (Gulati *et al.,* 2013).

The White Blood Cells can be classified into granulocytes and agranulocytes based on their light scatter(size) and absorbance characteristics analyzed. Granulocytes include neutrophil, basophil and eosinophil while agranulocytes include monocyte and lymphocyte. Absolute cell count and percentages are reported for each cell type categorized. The morphology flags indicate the presence of left shift (LS), immature granulocytes, large platelets, abnormal clustering, blasts, nucleated red blood cells etc. Elevated white blood cell count indicate infection, inflammation and some forms of cancer or leukemia in dogs (Bradfute, 2007).

Selective manual differential counts may improve efficiency, economy, and safety while not compromising patient care. It is easily adaptable to many situations, making it highly versatile. The observer can defect the clinically significant changes in the appearance of white blood cells (Ravin & Loy, 2016). Correcting entries is easier, too. There are different parasitic infections that can be seen in canines and these infections can be associated with different hematological characteristics. Identification of hematological alterations during routine laboratory screening of blood samples from dogs displaying clinical signs is essential for diagnosing blood parasitic infections (Honda *et al.,* 2016).

Tick-borne pathogens, including protozoans, bacteria and viruses, can cause serious illnesses in both humans and domestic animals. Dogs infected by different tick-borne pathogens typically present with similar clinical signs such as high fever, drowsiness, loss of appetite, pale mucous membranes, vomiting, and weight loss. The diagnosis of tick-borne diseases is performed usually based on the observation of clinical signs in conjunction with laboratory testing (Koepke *et al.,* 1985). Microscopic examination of blood smears is the conventional and routine diagnostic method, as it allows the identification of blood parasites based on their morphology. This technique is not expensive and detects acute infections successfully (Thongsahuan, 2020). The present study also aimed to compare the hematological profiles between infected and healthy dogs to identify hematological alterations caused by different parasitic, viral and bacterial infections.

2. Methodology

2.1 Samples for the study: The present study was conducted in a private pet clinic situated at Pala, Kottayam during the month of February 2022. Samples for the study constituted

blood from 5 dogs in their diseased and recovered condition.

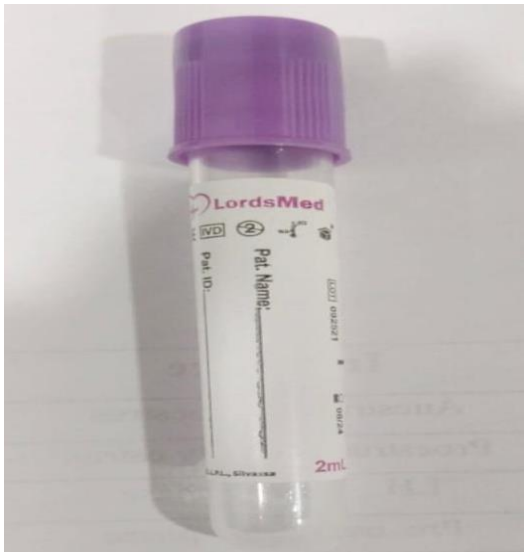
2.2 Collection of blood: 2 ml of blood was collected in an EDTA vial (Fig :1) using scalp vein set(20g) (Fig: 2) and syringe from the cephalic vein(forelimb) of dog. A small portion was subjected to complete blood count analysis in CBC analyzer(Huma count 30TS)(Fig:3). Also, blood smear was prepared from the same collected blood.

2.3 Preparation of blood smear: Blood smear was prepared by placing a small drop of blood on a pre-cleaned labelled slide at one end. Then brought another slide at an angle of 30°-40° upto the drop allowing the drop to spread along the contact line of two slides. Then quickly pushed the upper slide towards the other end of lower slide. Selected those smears having good feathered edges. Dried it and fixed with methanol.

2.4 Staining

- The slide with blood smear was kept on a staining rack.
- Prepared 1/10 dilution of Giemsa stain (Fig:4) by taking 1-part stock solution and 9-part distilled water.
- Poured the diluted Giemsa solution on to the blood smear and kept for 45 minutes.
- Poured off the stain and washed in running water until the stain ceases to come out.
- Blot the smear air dried and observed under microscope.

2.5 Microscopic examination of blood: Took the stained blood smear and observed under microscope. White blood cells are first observed under low power magnification. Then placed a small drop of cedar wood oil over the blood smear. The oil immersion objective was brought down just to touch the drop of oil on the smear. Once it touched the oil drop worked with the fine adjustment alone so that the smear was actually brought to focus. Began counting of different types of cells based on morphological characteristics (Table-1). Proceeded counting from left hand corner top position to right covering to entire smear. Then pushed the slide upward proceeded to left. This way the entire smear was brought under focus without duplicating the counting of cells. Counted upto 200 WBC's and calculated the percentage of different type of leukocyte in the blood smear.



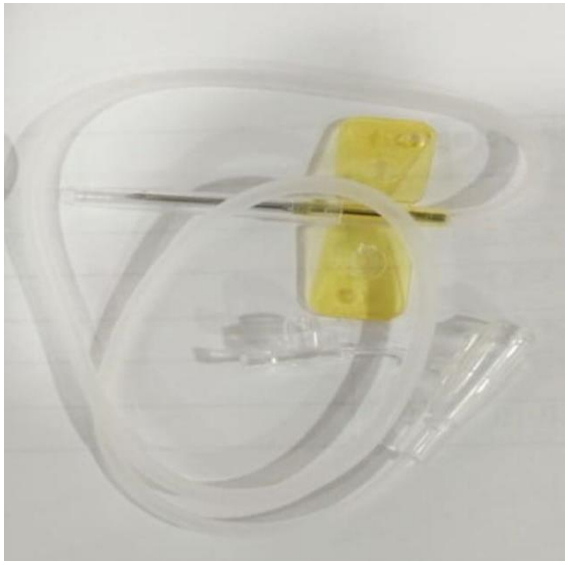
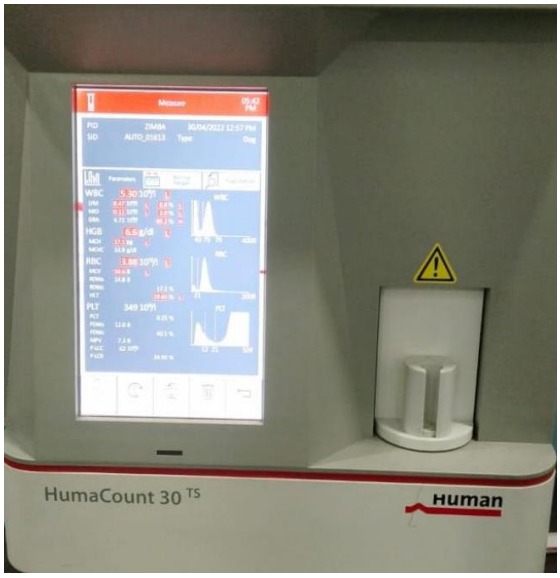


Fig. 1 EDTA vial **Fig. 2** Scalp vein set(20g)



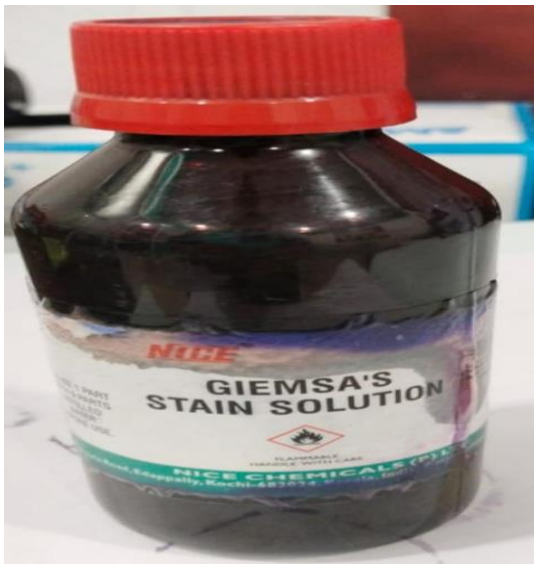


Fig. 3 Huma count 30TS**Fig. 4** Giemsa stain

Table 1: Morphological and staining characteristics of different Leukocytes

Blood Cell	**Morphological characteristics**	**Staining characteristics**
Neutrophil	Multi-lobulated	Blue coloured nucleus (4-5 lobes). Cytoplasm contain fine neutral coloured granules.
Eosinophil	Small size, round shape,	Pale blue cytoplasm, round to oval, brick red to pale blue

	bilobed nucleus.	granules.
Basophil	Small size, round shape, unlobed nucleus.	Pale blue cytoplasm, variable number of small medium and large dark red-purple granules.
Lymphocyte	Small to medium size, typically round to triangular shape, centrally positioned large round nucleus.	Pale blue cytoplasm.
Monocyte	Large size, typically round shape, kidney shaped nucleus.	Cytoplasm pale blue to pale gray.

3. Results & Discussion

The present study was carried out to show the difference in blood values of the diseased and recovered dog. The study also compares the result of differential leukocyte count obtained by machine method and manual method. This work demonstrated that how various types of infections affect the health of dogs (Table- 2 & 3)

Table-2 Blood analysis of dog-Before treatment

Name of dog	Breed	DLC obtained by manual method	Complete blood count obtained by CBC analyzer	Tentative diagnosis
Ziya(dog1)	Lab	N-51% E-14% M-3% L-32%	Total WBC- 20.68×10^9/l L-36.9% M+E+B-4.6% N-58.6% Hb-3.4g/dl RBC- 1.2×10^12/l PLT-750000	Babesiosis (parasitic disease)
Bella(dog2)	Labrador retriever	N-80% E-4% M-4% L-12%	Total WBC- 20×10^9/l L-28% M+E+B-3.9% N-62.1% Hb-14 RBC- 6.2×10^12/l PLT-250000	Bacterial Pneumonia
Alexy(dog 3)	GreatDane	N-90% E-4%	Total WBC- 72×10^9/l	Bacterial Myositis

		M-1% L-5%	L-22% M+E+B-2% N-90% Hb-13.2 RBC- 5.8×10^12/l PLT-218000	
Rambo(dog 4)	German Shepherd	N-60% L-8% E-2% M-2%	Total WBC- 2.3×10^9/l L-29.3% M+E+B-3.5% N-67.2% Hb-12.5 RBC- 5.9×10^12/l PLT-243000	Parvoviral enteritis
Turbo (dog 5)	Shih Tzu	N-82% E-6% M-4% L-8%	Total WBC- 4.2×10^9/l L-6.2% M+E+B-1.8% N-62% Hb-11.8g/dl RBC- 7.81×10^12/l PLT-215000	Canine distemper

[*N- neutrophil, E-eosinophil, B-basophil, M-monocyte, L-Lymphocyte, Hb-haemoglobin, PLT-platelet]

Table-3 Blood analysis of dog-After treatment

Dog	DLC obtained by manual method	Complete blood count obtained by CBC analyzer
Dog 1	N-62% E-10% M-4% L-30%	Total WBC-12.93×10^9/l L-40.6% M+E+B-7.4% N-52% Hb-7.6g/dl RBC-3.12×10^12/l PLT-139000
Dog2	N-77% E-6% M-4% L-13%	Total WBC-15.93×10^9/l L-18% M+E+B-2.8% N-68% Hb-16g/dl RBC-7.2×10^12/l PLT-318000
Dog 3	N-82% E-6% M-3%	Total WBC-20×10^9/l L-20% M+E+B-4%

	L-9%	N-68% Hb-10.8 g/dl RBC-4.6×10^12/l PLT-248000
Dog 4	N-64% L-14% E-8% M-3%	Total WBC-5.4×10^9/l L-29.5% M+E+B-3.8% N-30% Hb-11.2g/dl RBC-6.2×10^12/l PLT-302000
Dog 5	N-67% E-6% M-2% L-25%	Total WBC-9.5×10^9/l L-27.9% M+E+B-3.9% N-72% Hb-14.02g/dl RBc-6.81×10^12/l PLT-368000

[*N- neutrophil, E-eosinophil, B-basophil, M-monocyte, L-Lymphocyte, Hb-haemoglobin, PLT-platelet]

Honda *et al.* (2016), stated that if WBC count is constant, the presence of left shift indicates an increase of neutrophil consumption that is equal to an increase of production. A decrease in WBC count indicates that neutrophil consumption surpasses supply. During a bacterial infection, large numbers of neutrophils are consumed. The present work also reveals that there is elevated level of neutrophil during bacterial infection.

Bradfute (2007), suggested that analysis of White Blood Cells (WBC's) by flow symmetry showed a gradual but profound increase in percentage of granulocytes and a decrease in percentage of lymphocyte during the viral infection. The present work confirms the above study because there is a decrease in the level of lymphocyte during viral infection.

Behm & Ovington (2000), states that eosinophilia – an increase in the number of eosinophils in the blood or tissues – has historically been recognized as a distinctive feature of helminth infections in mammals. Eosinophils are also responsible for considerable pathology in mammals because they are inevitably present in large numbers in inflammatory lesions associated with helminth infections or allergic conditions. The present work stipulates with the above work that during parasitic infection there is an elevated level of eosinophil.

4. Conclusion

The present study concluded that the result obtained by manual method almost matches with that of automated DLC method. This study also confirms that increased level of White Blood Cells (WBC) in blood samples indicates the presence of infection or inflammation in the body because the body release more of these cells to fight against the infection. This work shows that there is an elevated level of neutrophil during the bacterial infection of dog (Dogs- Bella and Alexy). Viral infections can give rise to

a systemic decrease in the total number of lymphocytes in the blood referred to as lymphocytopenia. There is a further increase in level of eosinophil during parasitic infections (Dog-Ziya). There is no doubt that the findings of the present study would encourage the people to pay attention towards the general health of their pets.

5. Acknowledgements

The authors are thankful to the Principal and Head of the Department of Zoology, Alphonsa College, Pala, Kottayam for providing necessary facilities and consistent encouragement to complete the study successfully.

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