UNIVERSITI PUTRA MALAYSIA

HAEMATOLOGICAL STUDY OF CAPRINE SUBCLINICAL AND CLINICAL MASTITIS

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HAEMATOLOGICAL STUDY OF CAPRINE SUBCLINICAL AND CLINICAL MASTITIS

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It is hereby certified that we have read this project paper entitled "Haematological Study Of Caprine Subclinical and Clinical Mastitis'', by Nurul 'Atiqah binti Khairudin and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement of the course VPD 4999 – Project.

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DEDICATION

This work is dedicated to my beloved parents and family for their endless love, patience and support.

My parents, Khairudin Mohamad, Hasmah Osman, Norazizah Aripin and family members, Hussaini, Ruqaiyah, Najihah, ‘Ufairah, Iqbal, Qays and Ihsan.

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LIST OF ABBREVIATIONS

CMT	California Mastitis Test
NAGase	N-acetyl-β-D-glucosaminidase
SCC	Somatic cell count
EC	Electrical conductivity
RBC	Red blood cell
Hgb	Haemoglobin
PCV	Packed cell volume
MCV	Mean corpuscular volume
MCHC	Mean corpuscular haemoglobin concentration
WBC	White blood cell

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek.

KAJIAN HEMATOLOGI SUBKLINIKAL DAN KLINIKAL MASTITIS KAMBING

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Mastitis adalah salah satu penyakit yang memberi kesan kepada ekonomi yang melibatkan ladang tenusu seluruh dunia. Analisa hematologi adalah salah satu kaedah diagnostik yang boleh memberikan maklumat yang signifikan, tambahan kepada pemeriksaan fizikal. Tujuan kajian ini adalah untuk membandingkan parameter hematologi dalam tiga kumpulan kambing yang berbeza; sihat, subklinikal mastitis dan mastitis. Sampel darah telah diambil daripada 45 ekor kambing betina menyusu baka campuran; 16 kambing sihat, 16 kambing bersubklinikal mastitis dan 13 kambing berklinikal mastitis, daripada Ladang Angkat, Fakulti Perubatan Veterinar. Sampel darah diuji untuk parameter hematologi berlainan termasuklah PCV, hemoglobin, jumlah kiraan sel darah merah, jumlah kiraan sel darah putih, jumlah mutlak sel darah putih dan indeks MCV dan MCHC.

Parameter hematologi untuk semua kumpulan telah dibandingkan dengan analisis statistik. Keputusan menunjukkan tiada perbezaan signifikan ($p>0.05$) di antara semua nilai hematologi di bagi setiap kumpulan. Walau bagaimanapun, untuk kiraan keseluruhan sel darah putih kambing betina berklinikal mastitis signifikan ($p<0.05$) lebih tinggi daripada kambing betina sihat, ini menunjukkan sedang berlaku tindak balas imun semasa mastitis. Leukositosis adalah satu indikasi status inflamasi seperti inflamasi akut dan kronik dan juga stres. Faktor lain yang perlu diambil kira adalah baka, adaptasi fisiologi dan peringkat laktasi yang boleh menyebabkan kepelbagaian dalam nilai hematologi. Analisis hematologi hendaklah disokong oleh kaedah diagnostik yang lain seperti kiraan sel somatic (SCC), pemeriksaan bakteria dan konduktiviti elektrik pada susu untuk mendiagnos mastitis.

Kata Kunci: subklinikal mastitis, klinikal mastitis, kambing, kajian hematologi

ABSTRACT

An abstract of the project presented to Faculty of Veterinary Medicine in partial fulfillment of the course VPD4999- Project.

HAEMATOLOGICAL STUDY OF CAPRINE SUBCLINICAL AND CLINICAL MASTITIS

By

Nurul 'Atiqah Khairudin

2015

Supervisor: Dr. Rozaihan Mansor

Mastitis is an important economic disease affecting dairy farms worldwide. Haematological analysis is one of the diagnostic procedures that can provide significant information, additional to that resulting physical examination attributes. The purpose of this study is to compare the haematological parameters in three different groups of lactating does; healthy, subclinical mastitis and clinical mastitis. Blood samples were collected from 45 cross breed does; 16 healthy, 16 subclinical mastitic and 13 clinical mastitic does, from three farms of Ladang Angkat, Universiti Putra Malaysia. Blood samples were examined for different haematological parameters such as packed cell volume (PCV), haemoglobin (Hgb), and total red blood cell (RBC) count, total white blood cell (WBC) count, absolute white blood cell count as well as the mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC).

The haematological parameters for all groups were compared statistically. The results revealed there was no significant difference ($p>0.05$) for all haematological parameters of all the groups. However, the total white blood cell counts in clinical mastitic does were significantly ($p<0.05$) higher than that of the healthy does, which suggest an on-going immune response during mastitis. Leukocytosis is an indicator of the inflammatory status such as acute and chronic inflammation as well as during stress. Other factors should be considered such as breeds, physiological adaptation and lactational stage that may cause variations in the haematological values. Haematological analysis should be supported by other diagnostic methods such as somatic cell count (SCC), bacteriological examination and electrical conductivity of the milk to diagnose mastitis.

Keywords: subclinical mastitis, clinical mastitis, goats, haematological study

1.0 INTRODUCTION

1.1 Background of the study

Generally, there is an increase in the demand for goat milk due to an increase in society affluence and the traditional beliefs on health benefits of goat milk. However, there are challenges in dairy goat farming in Malaysia. According to Sithambaram and Nizam (2014), challenges in dairy goat farming include agricultural land, cost of feed, environment, marketing and disease. In this study, the focus will be on the most common diseases in dairy goats; mastitis. It is an economically important disease of small ruminants that is associated with decrease in milk production (Contreras et al., 2003). Previous studies on mastitis were done, especially subclinical mastitis as it is difficult to be diagnosed compared to clinical mastitis due to the absence of any visible indications (Viguier et al., 2009). The commonly used diagnostic methods for detection of subclinical mastitis are bacterial culture, which is the "gold standard" technique for the determination of udder health status, somatic cell count (SCC), California Mastitis Test (CMT), electrical conductivity of milk (EC) as well as milk composition (Sthur and Aulrich, 2010). Haematological studies can be used as diagnostic tool in mastitis, and a study done by Ajuwape et al. (2005) had compared the haematological values between non-mastitic and also clinical mastitic does. However, the haematological values comparison between non-mastitic, subclinical and clinical mastitic does has not been conducted in any studies.

1.2 Objective of the study

To compare the haematological values in three different groups of goats (healthy, subclinical and clinical mastitis).

1.3 Hypotheses of the study

H_{o1}: There is no significant difference in haematological response in goats affected by mastitis in comparison to healthy animals.

H_{A1}: There is a significant difference in haematological response in goats affected by mastitis in comparison to healthy animals.

H_{o2}: There is no significant difference in haematological response in goats affected by subclinical and clinical mastitis.

H_{A2}: There is a significant difference in haematological response in goats affected by subclinical and clinical mastitis.

2.0 LITERATURE REVIEW

2.1 Mastitis

2.1.1 Subclinical and clinical mastitis

Mastitis is (mast = breast; itis = inflammation), the inflammation of the mammary gland (Viguier et al., 2009), and it can be subclinical or clinical mastitis. Subclinical mastitis is the most common form of mastitis where there is no gross inflammation of the udder and gross changes in milk with decreased in production and lowered milk quality (Andrew, 2009). Subclinical mastitis is difficult to diagnose due to the absence of any visible indications (Viguier et al., 2009). Clinical mastitis can be classified into peracute, acute, subacute and chronic where there is a gross inflammation of the udder. Changes in milk composition such as fat, protein, lactose, and casein also occur during mastitis as reported by (Leitneret al., 2004). Other than that, mastitis also will cause changes including raised water content and the presence of visible clots and flakes in the milk (Viguier et al., 2009).

2.1.2 Aetiology of mastitis

Contagious pathogens that cause contagious mastitis are *Streptococcus agalactiae, Streptococcus dysgalactiae, Staphylococcus aureus* and *Mycoplasma sp*. agents are spread by milking procedures, contaminated machinery. There are also environmental pathogens that cause mastitis. These pathogens normally will not affect the mammary gland unless there are contamination of the environment, teats, udder or milking machine. According to Moroni et al. (2005), *Staphylococcus epidermis* has the highest percentage in intramammary infection in goats with 36.1%, followed by *S. chromogenes* 16.6% and *S. aureus* 7.1%.

2.1.3 Diagnostic methods

There are several diagnostic methods that can be used to diagnose mastitis. Clinical examination of the udder is an important step during physical examination on mastitic animals as any udder abnormalities can be detected such as warm and swollen udders (Fragkou et al., 2014). One of the oldest and best known diagnostic methods is the California Mastitis Test (CMT) (Schalm et al., 1957). The cells from milk are lysed after the detergent is added releasing nucleic acids and other constituents which lead to the formation of a 'gel-like' matrix consistency. CMT will roughly estimate the number of cells of the immune system and epithelial cells from the milk sample (Schaeren & Maurer, 2006). Recently, CMT can be classified into scores that range on a scale of 0

(negative), 1, 2 or 3 (Höhn, 2006). According to (Pyorala, 2002), indicators of inflammation such as mastitis can be used to detect mastitis. Milk somatic cell count (SCC) is one of the most common diagnostic methods used based on proportion of neutrophils as percentage of the SCC. Detection of enzymes such NAGase has also been proven as one of the diagnostic methods. Furthermore, electrical conductivity (EC) of milk is also used in diagnosing mastitis in small ruminants, based on the ionic changes of sodium and chloride concentrations increases during inflammation (Hillerton and Semmens, 1999). However, the potential of EC to be used in goats in monitoring mastitis is still inconclusive (Tanggora et al., 2010).

2.2 Complete Blood Count

2.1.2 Erythron

Red blood cells (RBCs) are produced primarily by the kidneys in response to erythropoietin. A complete evaluation of RBCs should include a-packed cell volume (PCV) or haematocrit (HCT), RBC count, haemoglobin (Hgb), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC). PCV will determine the percentage of RBCs per volume of blood, where it is the result of maximal packing of the erythrocytes after being measured in a column of blood after centrifugation (Weiser, 2012). Hgb represents the capacity of the red blood cell to carry oxygen throughout the body.

MCV indicates the average cell size while MCHC represents the average cell Hgb concentration (Jones and Allison, 2007). MCV and MCHC can be calculated using formulas:

Figure 1: Formula of MCV

$$\text{MCV (fL)} = \frac{\text{PCV(decimal fraction)} \times 1{,}000}{\text{RBC}(\times 10^{12}/\text{L})}$$

Figure 2: Formula of MCHC

$$\text{MCHC (g/L)} = \frac{\text{Whole blood hemoglobin concentration (g/L)}}{\text{PCV(decimal fraction)}}$$

Normal values for erythrocytes parameters in goats are presented in Table 1.

Table 1: Normal erythrocytic values for goats

Erythron	**Range**
Erythrocytes ($\times 10^6/\mu$L)	8.0 – 18.0
Hemoglobin (g/dL)	8.0 – 12.0
PCV (%)	22 – 38
MCV (fL)	16 – 25
MCHC (%)	30 – 36

2.1.3 Leukon

Leukocytes consist of granulocytes and mononuclear cells. Granulocytes includes neutrophils, eosinophils and basophils while mononuclear cells includes lymphocytes and monocytes (Jones and Allison, 2007). Neutrophils will migrate

into damaged tissue within 2 hours of an insult for phagocytosis of foreign material and bacteria (Morris, 2002) while eosinophils play an important role in the immune response towards parasites, allergens, and other inflammatory processes. Basophils in goats are usually small in numbers, and they function in allergic and inflammatory processes by releasing inflammatory mediators. Monocytes which then be converted into macrophages, has the capability to phagocytize infectious organisms, particulates and cell debris. Increases in monocytes may indicate chronic inflammation, tissue necrosis, or stress response. While lymphocytes are responsible for innate immune response (Michael, 2010).

Normal values for leukocytes parameters in goats are presented in Table 2

Table 2: Normal leukocytic values for goats

Leukon	**Range**
Total leukocytes (/ μ L)	4,000 – 1,300
Neutrophil (band)	Rare
Neutrophil (segmented)	1,000 – 7,200
Lymphocyte	2,000 – 9,000
Monocyte	0 – 550
Eosinophil	50 – 650
Basophil	0 – 120

3.0 MATERIALS AND METHODS

3.1 Animals selection

A total of 45 lactating does of cross breeds were selected in this study. California Mastitis Test (CMT) and the presence of clinical mastitis signs were used to determine the health status of our selected animals.

A total of 16 healthy lactating does (CMT result <1+, no mammary gland abnormalities, no milk abnormalities, clinically healthy animals) with another 16 lactating does with subclinical mastitis (CMT result >1+, no mammary gland abnormalities, no milk abnormalities) were selected. Thirteen lactating does with signs of mastitis (warm, painful and swollen udder and/or milk discoloration, milk with flakes, clots) were selected and grouped as does with clinical mastitis.

3.1.1 California Mastitis Test (CMT)

CMT was done where the teats were massaged and applied with alcohol swab, and the early stream of milk was removed. Two ml of milk was milked on the CMT paddle, and three ml of CMT reagent added and mixed with the milk. The texture of the milk was assessed within 10 seconds and scored.

3.2 Collection of blood samples

Approximately two ml of blood were collected from jugular vein using vacutainer into ethylenedinamine tetra-acetic acid (EDTA) tube and the blood samples were transported in an ice box to Haematology and Clinical Biochemistry Laboratory at Faculty of Veterinary Medicine, Universiti Putra Malaysia.

3.3 Haematological analysis

Packed cell volume (PCV) was determined by hematocrit method and estimated using Hawksley haematocrit tube reader, while haemoglobin (Hgb) concentration, red blood cell and white blood cell counts and mean corpuscular haemoglobin concentration (MCHC) were determined using automated haematology analyzer machine (CELL-DYN 3700) and mean corpuscular volume (MCV) was determined using formula. Differential leukocytes counts were calculated manually under microscope (40x magnification).

3.4 Statistical analysis

Statistical analysis of data was carried out using Kruskal-Wallis as described by Pallant (2005) to determine the significant difference of all parameters of the different groups. Mann-Whitney test was carried out to compare parameters with significant values in different groups. Mean and standard error of the different parameters were also determined.

4.0 RESULTS

4.1 Red blood cell parameters

Haematologically, the erythrocytic indices of all groups; healthy, subclinical and clinical mastitis were not significantly different ($p>0.05$). The mean RBC, Hgb, PCV, MCV and MCHC for all groups are shown in Table 3. RBC indices parameters in all groups are within normal range.

Table 3: Mean of PCV, Hgb concentration, RBC, MCV and MCHC of healthy, subclinical and clinical mastitis.

Mastitis status	RBC($x10^{12}$/L)	Hgb(g/L)	PCV (%)	MCV	MCHC
Healthy (n= 16)	12.1 ± 0.19	85.1 ± 1.99	26.1 ± 3.23	21.7 ± 0.62	329.1 ± 7.94
Subclinical mastitis (n=16)	11.6 ± 0.45	85.2 ± 3.57	25.3 ± 4.39	21.9 ± 0.52	338.4 ± 9.62
Clinical mastitis (n=13)	12.2 ± 0.29	88.8 ± 2.5	25.9 ± 3.95	21.2 ± 0.50	345.7 ± 8.03

Data expressed as mean±standard deviation

4.2 White blood cell parameters

The total white blood cell counts and differential absolute white blood cell counts of the mean of total and differential white blood cell counts for all groups are shown in Table 4. The total WBC of clinical mastitic does was increased in comparison to the normal range of WBC which is suggestive of leukocytosis and served as the highest value

among the three different groups. All lactating does had an increased value of band neutrophils, however the values for segmented neutrophils were within normal range. Statistically, the value of total white blood cell counts of clinical mastitic does was significantly ($p<0.05$) higher than healthy does. The presence of band neutrophils in the blood was suggestive of left shift leukocytosis. Lymphocytes counts were found within normal range, with the clinical mastitic does had the highest value followed by subclinical mastitis and healthy group. However, healthy does had an increase of their monocytes values, suggestive of monocytosis. Additionally, eosinophils and basophils values were found to be within normal range for all the three groups.

Table 4: Mean of total and differential white blood cell counts of healthy, subclinical and clinical mastitis.

	WBC ($x10^9$)	**Band Neutrophils ($x10^9$)**	**Neutrophils ($x10^9$)**	**Lymphocytes ($x10^9$)**	**Monocytes ($x10^9$)**	**Eosinophils ($x10^9$)**	**Basophils ($x10^9$)**
Healthy (n=16)	10.5 ± 0.47^{a}	0.6 ± 0.20	4.6 ± 0.48	4.1 ± 0.42	0.7 ± 0.12	0.3 ± 0.05	0.00
Subclinical mastitis (n=16)	11.8 ± 0.69^{ab}	0.7 ± 0.20	3.2 ± 1.10	4.8 ± 1.16	0.5 ± 0.13	0.1 ± 0.07	0.0 ± 0.02
Clinical mastitis (n=13)	13.3 ± 0.84^{b}	0.3 ± 0.08	7.0 ± 1.4	5.3 ± 1.4	0.5 ± 0.18	0.6 ± 0.42	0.00

Data expressed as mean±standard deviation, ab means with column with different superscripts are significantly different at $p<0.05$.

5.0 DISCUSSION

5.1 Red blood cell parameters

In this study, the erythrocytic values obtained for healthy, subclinical and clinical mastitis does fell within normal range as reported by (Byers and Kramer, 2010). Based on Ajuwape et al., (2004), there was significantly ($P < 0.05$) lower PCV, Hgb concentration, RBC count, MCHC and MCV in mastitic does compared to the non-mastitic does. The lower values of these parameters indicated that bacterial mastitis caused depression of erythrocytic indices. However, in this study there are no significant ($p>0.05$) difference between all of the erythrocytic indices among all of the three groups. The mean of haemoglobin (Hgb) in clinical mastitic does was the highest, followed by subclinical mastitic and healthy does. While the mean of PCV in healthy does was higher than in the subclinical and clinical mastitic does, however they were within normal range. This can be suggestive of the depression of erythrocytic indices due to the clinical mastitis. Other than that, MCV and MCHC were all within normal range.

5.2 White blood cell parameters

The mean value of total white blood cell counts of clinical mastitic does was significantly ($p<0.05$) higher than healthy does. The finding supports the observation by Ajuwape et al., (2004), in which the white blood cell counts in mastitic does were significantly ($p<0.05$) higher than healthy does as a result of leukocytosis in the clinical mastitic does. According to Jain, (1986), an elevation of white blood cells is an indicator

of acute tissue necrosis and endotoxin-induced response associated with septic mastitis. This finding suggests an on-going bacterial infection (mastitis). In healthy ruminant, band neutrophils are rarely found in blood (Byers and Kramer, 2010), however the result in this study revealed the presence of band neutrophils in all groups suggestive of inflammation. Regenerative left shift is signified by the increased of mature neutrophils in the addition to band neutrophils, indicative of inflammation of the mammary gland in does. Meanwhile, monocytosis in healthy group may be indicative of underlying chronic diseases or stress response other than mastitis, occurs in the healthy does, as monocytosis is an indicator of chronic inflammation, tissue necrosis, hemolysis, or a stress response. (Jones and Allison, 2007). In this study, the clinical mastitic does has higher mean of total WBC as compared to the subclinical mastitic and healthy does. WBC was not significantly different between clinical and subclinical mastitic does and subclinical and healthy does, suggestive of the three groups of does are in different states of inflammation, as well as different stress level.

CONCLUSION

There was no significant difference in haematological response for the three groups, except for total white blood cell. However, total white blood cell was significantly higher in lactating does with clinical mastitis compared to healthy lactating does. Haematological analysis reflects the systemic state of the animal but not as a definitive diagnostic method for mastitis. It should be supported by other diagnostic methods such as, somatic cell count (SCC) or bacteriological examination or electrical conductivity of the milk.

RECOMMENDATIONS

For further study for this project, it is recommended:

1. To combine this method with other confirmatory methods like bacterial isolation and identification or somatic cell count (SCC).
2. To compare the haematological parameters between different classifications of mastitis, such as the aetiologial agents or time frame (peracute, subacute, acute and chronic mastitis).

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