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# Hematological Parameters of sheep: An Aid in the Diagnosis of Gastrointestinal (GIT) and Respiratory Diseases

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**Abstract:** The present hematological study was conducted on sheep to assess the hematological parameters of apparently normal and diseased sheep as a diagnostic tool for GIT and respiratory diseases. A total of 36 blood samples were collected from 12 apparently normal sheep (6 from each sex) and 24 diseased ones. In the latter case, 12 sheep with GIT diseases were sampled comprising 6 sheep from each sex. Likewise, 12 sheep with respiratory diseases were sampled constituting 6 sheep from each sex. The blood samples collected were subjected to laboratory analysis to determine ESR, Hb, PCV, TEC, TLC and DLC. Erythrocytic indices were calculated from the values of Hb, PCV and TEC. In apparently normal sheep, the mean values of Hb, PCV and TEC were significantly higher in males than females and it was observed statistically significant (P<0.05). In GIT infected sheep as compared to apparently normal ones, the mean values of Hb, PCV, TEC, MCHC and Lymphocyte in males were found to be decreased, where as ESR and TLC in females were significantly increased (P<0.05). The mean values of TLC and neutrophils in males with respiratory infections were significantly increased where as lymphocytes were decreased as compared to apparently normal ones. The values of ESR, TLC, eosinophils and monocytes were significantly higher (P<0.05) in diseased females than the apparently normal ones. In similar condition, the mean values of TLC and Neutrophils in diseased males were significantly increased (p<0.05), where as lymphocytes were decreased when compared with apparently normal males.

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**Key words:** *GIT infections; Hematological parameters; normal conditions; Respiratory infections; Sheep*

## 1. Introduction

Sheep are small ruminants that have special attributes over other livestock resources. They are more adapted to broad ranges of environment, have short generation cycles and reproductive rate which lead to high production efficiency and poor people can afford them with less cost (Seare, 2007). These animals represent an important component of Ethiopia livestock production system, providing 12% of the value of livestock products consumed at the farm level and 48% of the cash income generated (FAO, 1983). In Tigray region, the population of sheep is estimated to be 935,349 and in Mekelle it could be estimated to 2798 (BoANR, 2005). According to BoANR, in this region there are four breeds of sheep namely: Degua, Abergalle, Begait and Elle. However, they are among the domestic animals that are commonly suffering from a number of disease conditions (Gillespie, 1992).

The hematological examination provides useful information in assessing the health status of various animals' species. The red blood cells, also known as erythrocytes, and white blood cells (leukocytes) are an important medium for diagnosing diseases (Kelly, 1973).

Therefore, hematological analysis of blood is a powerful diagnostic tool. Veterinary technicians provide a valuable service by acquiring the skills necessary to perform this analysis. Both the physiological and pathological conditions of the animals can be assessed by examination of hematological and biochemical analysis of blood (Coles, 1986). There are different possible factors such as diseases; nutrition, sex, breed and other clinical conditions affect the hematological and biochemical parameters of apparently healthy animals (Awah and Nottdge, 1998).

Hematological examinations are performed on un clotted blood samples, but in order to secure reasonable accuracy suitable anticoagulants must be used. The hematological changes are mostly in the form of either increase or decrease of various blood constituents. A significant increase in erythrocyte number occurs in

polycythaemia and disturbed tissue fluid balance such as dehydration and other similar derangements of tissue fluid balance give rise to elevated erythrocyte counts because of a decreased in circulating plasma volume (Kelly, 1973).

Routine hematological testing in the laboratory could include determination of erythrocyte sedimentation rate (ESR), hemoglobin concentration (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC) and differential leukocyte count (DLC) (James and Dennis, 2000).

Therefore, the objectives of this study were to compare the hematological parameters of diseased and apparently normal sheep as a diagnostic tool for GIT and respiratory diseases.

## 2. Materials and Methods

**Study area:**

The study was conducted in Mekelle town, the capital city of Tigray regional state, Ethiopia. It is geographically located in between 33º 24′ 30″ to 13º 36′ 52″ latitude and 39º 25′ 30″ to 39º 38′ 33″ longitudes. It lies in altitudinal range of 2150 -2270 m above sea level. The average temperature of the town is 11.11ºc to 24.1ºc per year, but some studies show there is high temperature fluctuation.

**Study Design and Study Population:**

The study was conducted to evaluate the different hematological parameters of the diseased and apparently normal sheep. Animals (particularly those supposed to be diseased) were selected by purposive sampling. The apparently normal animals could also be selected for comparison of the different hematological values with that of the diseased animals. The sheep for the study were those presented to the clinic manifesting clinical conditions of respiratory and GIT infections. They were grouped based on the symptoms and sex categories. Accordingly, a total of 36 sheep were included in the study consisting of 12 apparently normal sheep (6 from each sex) and 24 diseased sheep. In the latter case, 12 sheep with GIT diseases were sampled comprising 6 sheep from each sex. Likewise, 12 sheep with respiratory diseases were sampled constituting 6 sheep from each sex.

**Study Methodology and Procedures**

***Blood Sample collection*:**

Whole Blood samples were collected from animals, not being treated with any antibiotic and anthelmentic drugs, at an appropriate site of the vein (jugular vein) of the animals. The blood (5ml) was collected into vacutainer tubes which contained 0.5 ml of Ethylene diamine tetra acetate (EDTA) and the collected blood was gently mixed by slow rotatory movements. The blood smear was made from the fresh sample on a clear, dry and non-oily slide and fixed with methanol for 3-5 minutes for differential leukocyte count (Coles, 1986).

***Hematological Parameter Determination procedures:***

***Total Red Blood Cells:***

The total red blood cells and white blood cells count were performed by Haematocytometer method. For RBC the blood sample collected with anticoagulant was carefully mixed by swirling movement of the vial. The sample was drawn by erythrocyte diluting pipette (identified by the 101 mark above the bulb and red color of bead in it) exactly to the 0.5 mark using gentle suction on the mouth piece and excess blood past the line was expelled by stroking the tip of the pipette with the finger. The diluting fluid (isotonic salt solution) was drawn in to the pipette up to 101 mark above the bulb and the pipette was shaken for 3 minutes holding horizontally between the thumb and middle finger. The fluid filled the space between the counting chamber and the cover glass and then the hemocytometer was charged with fluid. Under high power magnification of microscope, all the erythrocytes in 5 of the 25 small squares in the central area were counted. Each of the 5 small squares contains other 16 smaller squares and a total of 80 of these small squares were counted (Schalm *et a*l., 1986).

***White Blood Cells Count***:

Similar method was applied for WBC count, but some differences are indicative. In this case the sample was drawn by leucocyte diluting pipette (identified by 11 mark above the bulb and white color of bead in it) exactly to the 0.5 mark and the blood was diluted by WBC diluting fluid to the 11 mark above the bulb so that 1:20 dilution was made. Number of cells in each of the four large corner squares was counted under low power magnification (x10) (Schalm *et a*l., 1986).

***Measuring Packed Cell Value:***

The hematocrit (packed cell volume, PCV) value was determined by using microhematocrit method. A capillary hematocrit tube was filled by the blood sample, containing anticoagulant, through capillary action. The opposite end of the tube was sealed by plastic caps, then the tube was placed in the centrifuge keeping the open end towards the hub and sealed end to the rim of the head and centrifuge for 5 minutes at 10,000 rpm. PCV was read by a hematocrit reader as percent (Benjamin, 1978).

***Hemoglobin Determination:***

Acid hematin method was used to determine hemoglobin (Hb) concentration using Sahli Helling's hemoglobinometer. The graduated tube of hemoglobinometer was filled with decinormal solution of hydrochloric acid to mark 10. The blood was sucked

using a clean and dry Hb pipette from the vial containing anticoagulant up to mark 20. The blood was expelled directly in to the graduated tube containing HCl acid solution. The content was mixed by swirling and allowed to stand for 8 minutes and then the mixture in the graduated tube was diluted with distilled water, drop by drop. The contents become mixed thoroughly with the stirrer after every addition so that the colour becomes matched with the standard. The corresponding reading was taken and the concentration of Hb was recorded as g/100ml of blood (Benjamin, 1978).

***Erythrocyte Sedimentation Rate Determination:***

Erythrocyte sedimentation rate (ESR) was determined by using Westergren method. The blood sample was drawn in to the tube up to the 0 mark and the tube was placed in an upright position in rack with rubber at the bottom so that the tube was sealed when inserted. The upper level of sedimenting erythrocytes was read on the left scale and the result was expressed as the fall of RBC in mm/hr (Benjamin, 1978).

***Differential Leukocyte Count:***

The blood smear was done by mixing blood sample properly by gentle agitation and then with the help of applicator stick, small drop of the blood placed near one end of the slide. Other slide was put on the slide with a drop of blood at an angle of 30$^{o}$ and the blood got spread with the spreader slide forward with a steady and even motion so that the blood made a thin film. The smear fixed with methanol was stained with Giemsa's stain to evaluate the differential leukocyte count. A total of 100 leukocytes were counted under oil immersion by using battlement method. This can be done through examination along the outer margin of the smear for about 3 fields, move in ward a short distance (3 fields), parallel the margin for 3 fields and then moved back to the edge of blood smear. The value for leucocytes was obtained by multiplying the percent by the total leucocyte count and dividing by hundred (Vahaneik, 1985).

***Measuring Erythrocytic Indices:***

Erythrocytic indices namely Mean corpuscular volume, (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC) were calculated from the values of RBC count, PCV percentage and Hb concentration by conventional method (Schalm *et al*., 1986).

***Data analysis*:**

The data were entered into a Microsoft excel spread sheets program and then was analyzed through SPSS version 17.0. The mean values, standard deviation and mean errors were calculated and the results were statistically done by using analysis of variance (two ways ANOVA). The parameters analyzed were ESR, Hb, PCV, TEC count, MCV, MCH, MCHC, TLC, neutrophils, eosinophils, basophils, monocytes and lymphocytes. All values were reported as statistical significant if p value was less than 5% (Steel and Torrie 1980; Cervenka, 1975).

## 3. Results

The present hematological study was conducted to determine the different hematological parameters in apparently normal and diseased (respiratory and GIT infections) sheep as an aid of diagnosing the diseases. The different hematological parameters presently evaluated were erythrocyte sedimentation rate (ESR), total erythrocyte count (TEC), hemoglobin concentration (HB), hematocrit determination (PCV), total and differential leukocyte count (TLC and DLC respectively). The values of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated from the values of HB, PCV and TEC. The results of these different hematological parameters of sheep are explained in tables 1 as follows.

**Sex-wise comparison of Hematological parameters in apparently normal sheep:**

Higher mean value of ESR was registered in males (5.5 $\pm$1.4mm/24hrs) than in females (5.0 $\pm$1.0 mm/24hrs); however, it was not statistically significant ($p > 0.05$). Higher mean value of Hb was recorded in males (13.4 $\pm$1.1 g/dl) than in females (8.5$\pm$ 0.9 g/dl), and this difference was statistically significant ($p < 0.05$). The study revealed that higher mean value of PCV was obtained in males (33.5$\pm$2.1%) than in females (25.2$\pm$1.4%). This variation was statistically significant ($p<0.05$). The mean value of TEC in males was 14.4$\pm$1.0x10 $^{6}$/µl and in females 7.5$\pm$1.1x10$^{6}$/µl. The variation indicated that there was statistically significant increase ($p<0.05$) in TEC in males when compared with females.

The mean value of MCV in males and females was 21.2$\pm$ 2.3 fl and 25.8$\pm$3.1fl respectively. However, there was no significant difference ($p > 0.05$). The mean value of MCH in males and females was 9.3$\pm$0.7 pg and 10.2$\pm$1.9 pg respectively; showing no significant differences ($p>0.05$).The mean value of MCHC in males was 40.9 $\pm$ 4.6g/dl and in females 36.2 $\pm$ 3.4g/dl. However, this value did not differ significantly. The mean value of TLC in males was 8.6 $\pm$ 1.8x10$^{3}$/µl, where as in females 7.4$\pm$1.5x10$^{3}$/µl. This showed that there was no statistical significant difference in both sexes ($p>0.05$). The mean values of DLC were 22.5$\pm$4.7%, 8.0$\pm$0.8%, 1.6 $\pm$ 0.5%, 4.0 $\pm$0.6%, 65.5$\pm$4.3% and 24.1$\pm$2.2%, 6.5$\pm$0.8%, 1.6$\pm$0.5%, 4.0$\pm$ 0.6% and 56.8 $\pm$ 2.4% for neutrophils, eosinophils, basophils, monocytes and lymphocytes in males and

females respectively. However, these values did not vary significantly in both sexes.

**Hematological parameters findings in clinically diseased sheep:**

***Respiratory infections:***

The study demonstrated that the mean value of ESR was higher in females (11.8±4.8mm/24hrs) than in males (9.8±3.1mm/24hrs). This finding revealed that the mean value of ESR was higher in diseased females' sheep than the normal ones (5.0 ±1.0 mm/24hrs). This increment was statistically significant ($p<0.05$). The mean value of Hb in males (9.6±1.6g/dl) and females (8.8±1.4g/dl) did not show any significant variation in both sexes as compared to apparently normal sheep. The mean value of PCV in males was 27.1±1.1% and in females 26.6±1.2%. Statistical analysis revealed that there was no significant change in PCV value. The mean value of TEC in males and females respectively was 13.0±3.9x$10^6$/µl and 10.9±3.5x$10^6$/µl. The value indicated that there was no significant change in TEC in diseased sheep as compared to the value of TEC in apparently normal ones.

The mean value of MCV in males was 19.9± 4.9fl and in females 25.9±7.0fl. Statistical analysis showed that there was no significant change of MCV value. The mean value of MCH in males and females was 7.2±1.0 pg and 8.6±3.2 pg respectively. However, there was no significant variation ($p>0.05$). MCHC's mean value in males and females was 35.2 ± 4.3g/dl and 32.9±4.0g/dl respectively; however, the study revealed that statistically significant difference was not registered in MCHC ($p>0.05$) in infected and apparently normal sheep. Higher mean value of TLC in males **(**16.7±4.1x$10^3$/µl**)** and females **(**12.3±1.2x$10^3$/µl**)** was registered in diseased sheep of both sexes than those of apparently normal ones. This indicated that statistically there was increase in TLC in diseased sheep when compared with apparently normal ones.

The mean values of DLC in males and females were 37.0±1.7% and 25.0±8.1%, 8.5±1.0% and 11.5±2.0%, 2.2±0.4% and 2.1±0.4%, 4.8±0.9% and 8.0±3.1%, 48.6±2.8 % and 53.3±8.7% for neutrophils, eosinophils, basophils, monocytes and lymphocytes respectively. The statistical analysis indicated that there was significantly increased neutrophils in infected males as compared to apparently normal males ($p< 0.05$) and inversely there was statistically significant increase ($p<0.05$) in eosinophils and monocytes in infected females as compared with apparently normal female sheep; but there was no statistical difference in basophiles ($p>0.05$) in infected and apparently normal sheep. Statistical analysis revealed that there was statistically significant decrease ($p<0.05$) in lymphocytes in diseased males when compared with apparently normal ones.

**GIT infections:**

Lower mean value of ESR was registered in males (7.6±1.0mm/24hrs) than in females (14.6±6.7mm/24hrs). This showed that there was statistically significant increase in ESR in females (diseased) when compared with the same sex of apparently normal animals. The mean value of Hb in males was 7.4± 2.6g/dl and females 6.9±1.1g/dl. Statistically Hb value was decreased in males when compared with apparently normal males. The mean value of PCV in males was 23.1± 4.6% and females 23.0±3.7%. There was significant decrease ($p<0.05$) in PCV in diseased males as compared to normal ones. The mean value of TEC in males and females was 8.6± 2.1x$10^6$/µl and 7.3±1.6 x$10^6$/µl respectively. Statistical analysis revealed that there was significant decrease in value of TEC in diseased males as compared to apparently normal ones.

Statistical analysis on the mean value of MCV in males (27.4±7.2fl) and females (31.9±3.6fl) did not reveal any significant change in diseased sheep as compared to apparently normal ones. It was also true for MCH in males (8.9±3.5pg) and females (9.7±2.5pg) had no significant differences as compared to apparently normal ones. The mean value of MCHC in both males and females was 32.3± 9.2 g/dl and 30.3± 6.0 g/dl respectively. There was significant decrease in MCHC in diseased males as compared to apparently normal ones ($p<0.05$). The mean value of TLC in males was 12.7±5.2x$10^3$/µl and in females 13.9± 3.9x$10^3$/µl; however, statistical analysis revealed that there was significant increased ($p<0.05$) in TLC in females as compared to apparently normal ones.

The mean value of DLC in males and females was 33.0± 5.4% and 32.3± 4.40 %, 8.2±1.9 % and 9.0±0.6%, 1.8±0.5% and 1.5±0.5%, 5.5±1.0% and 6.0± 0.6% and 50.5±1.3% and 51.2±4.7% for neutrophils, eosinophils, basophils, monocytes and lymphocytes respectively. Statistical analysis revealed that there was no significant differences observed in diseased animals for neutrophils, eosinophils, basophils, and monocytes; but a significant decrease $p<0.05$) in lymphocytes was observed in males when compared with apparently normal ones.

Table 1: Mean ±SD of different hematological parameters of apparently normal and diseased sheep

| Conditions | Sex | ESR /24hrs | Hb(g/dl) | PCV (%) | TEC ($10^6$/µl) | MCV(fl) | MCH (pg) | MCHC (g/dl) | TLC ($10^3$/µl) | N% | E% | B% | M% | L% |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Apparently normal | M(n=6) | 5.5±1.4 | 13.4±1.1 * | 33.5±2.1* | 14.4±1.2 * | 21.2±2.3 | 9.3±0.7 | 40.9±4.6 | 8.6±1.8 | 22.5±4.7 | 8.0±0.8 | 1.6±0.5 | 4.0±0.6 | 65.5±4.3 |
| | F(n=6) | 5.0±1.0 | 8.5±0.9 | 25.2±1.4 | 7.5±1.0 | 25.8±3.1 | 10.2±1.9 | 36.2±3.4 | 7.4±1.5 | 24.0±2.2 | 6.5±0.8 | 1.6±0.5 | 4.0±0.8 | 56.5±2.4 |
| GIT | M(n=6) | 7.6±1.0 | 7.4±2.6* | 23.1±4.6* | 8.6±2.1* | 27.4±7.2 | 8.9±3.5 | 32.3±9.2* | 12.7±5.2 | 33.0±5.4 | 8.2±1.9 | 1.8±1.0 | 5.5±1.0 | 50.5±1.3* |
| | F(n=6) | 14.6±6.7* | 6.9±1.1 | 23.0±3.7 | 7.3±1.6 | 31.9±3.6 | 9.7±2.5 | 30.3±6.0 | 13.9±3.9* | 32.3±4.4 | 9.0±0.6 | 1.5±0.5 | 6.0±1.0 | 51.2±4.7 |
| Resp. | M(n=6) | 9.8 ±3.1 | 9.6±1.6 | 27.1±1.1 | 13.5±3.9 | 19.9±4.9 | 7.2±1.0 | 35.2±4.3 | 16.7±4.1* | 37.0±1.7* | 8.5±1.0 | 2.2±0.4 | 4.8±0.9 | 48.6±2.8* |
| | F(n=6) | 11.8±4.8* | 8.8±1.4 | 26.6±1.2 | 10.9±3.5 | 25.9±7.0 | 8.6±3.2 | 32.9±4 | 12.3±1.2* | 25.0±8.1 | 11.5±2.0* | 2.1±0.4 | 8.0±3.1* | 53.3±8.7 |

SD=standard deviation, n=no. of animals, M=male, F=female, *=statistically significant
GIT= Gastrointestinal infections, Resp. =Respiratory infection

## 4. Discussions

The present study was conducted to determine the different hematological parameters of apparently normal and diseased sheep to aid in the diagnosis of disease conditions. The values of different hematological parameters of apparently normal sheep were compared sex-wise. In addition, hematological parameters were compared between clinically diseased sheep and apparently normal ones. However, concerning this study, there is no this much evident information on hematological studies of diseased sheep (respiratory and GIT infections) in our country still the present time.

In the present study, the mean values of ESR in apparently normal sheep didn't show significant differences in males and females. The mean values of Hb, PCV and TEC in apparently healthy sheep were higher in males than in females. This may be due to the activity of estrogen hormone on the erythropoietic processes which leads to relative decrease of RBC production (Coles, 1986). The study also showed that higher values of Hb, PCV and TEC were observed in males than the reports in West African male Dwarf Sheep ( Taiwol and Ogunsanmi, 1999); Olayemi *et al.*, 2000). This variation might be due to environmental, breed, feeding management and altitude factors.

This study revealed that the mean values MCH and MCHC were not differed significantly in males and females. However, these findings were higher than the earlier works reported in sheep kept under extensive management system (Taiwol and Ogunsanmi, 1999; Olayemi *et al.*, 2000). Besides, the mean values of Hb, PCV, and TEC in males in the current study were higher than the values of Hb and TEC reported for the local Menz sheep in central high lands of Ethiopia, North Shoa (Fufa, 1999). This might be due to different factors like environment, feeding management and others.

The mean values of TLC and DLC in males and females of apparently normal sheep were statistically insignificant. However, the mean values of TLC in males were in close agreement with the values of TLC reported for West African Dwarf Sheep managed under extensive management system (Olayemi *et al*., 2000).

The mean value of ESR in females affected with respiratory infections showed significant increase when compared to the ESR value in apparently normal ones. The significant change in ESR could be due to the abnormal morphological patterns of RBC and the increment of fibrinogen level in localized/ generalized infections (Gupta *et al*., 2003).

The mean values of Hb, PCV and TEC in sheep affected with respiratory infections did not show statistical significant variations when compared with apparently normal ones. In males infected with GIT infections, however, the values of Hb, PCV and TEC were significantly decreased as compared to apparently normal ones. The values of Hb and TEC show close agreement with the values of Hb and TEC reported for local Zebu cattle and influence of Trypanosomosis at Bedelle area (Yared, 2004)

It was observed that the mean values of MCV and MCH were not significantly changed in both males and females sheep affected with respiratory infections. The mean value of MCHC in females infected with GIT infections did not change significantly; however, it was decreased significantly in males. The value of MCHC obtained in the current study is similar with the value of MCHC reported for Arsi heifers and effect of natural exposure to sub clinical helminthiasis in Gobe Ranch (Girum, 1991).

This study showed the mean values TLC (in both sexes), eosinophils and monocytes in females, as well as neutrophils in males affected with respiratory infections were significantly increased; however, lymphocytes in males were significantly decreased when all compared with the values of TLC, eosinophils, monocytes and lymphocytes in apparently normal ones. This might probably be due to generalized or localized infections (Gupta *et al*., 2003).

In the present study, the values of TLC in females infected with GIT infections were significantly increased ($p<0.05$). Whereas, the values of lymphocytes in GIT infected males were significantly decreased when compared with apparently normal ones. These findings were in close agreement with TLC values reported for the local Arsi Heifers and effect of natural exposure to helmenthiasis in Gobe in Ranch (Girum, 1991).

## 5. Conclusions

The study conducted on different hematological parameters indicated that values obtained in apparently normal sheep were more or less similar and in the range of the values reported by different authors; even though, a little work had been done on the hematological study in these animals in Ethiopia. However, most of the erythrocytic parameters revealed higher values in males than females. The study showed some of the blood parameters of sheep were found to be changed/variable with the disease conditions (GIT and/or respiratory infections), either increase or decrease in some of hematological parameters when compared with the values obtained in apparently normal ones. The variations in hematological values can be helpful in diagnosing the disease conditions that influence physiological patterns of the blood cells. Therefore, it is recommended that further researches should be conducted on hematological parameters of

sheep to use it as a diagnostic tool in disease conditions in Ethiopia.

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