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Hematological Profile of Indigenous Sheep in Rajshahi Metropolitan Area of Bangladesh

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ABSTRACT

Hematological profiles are important candidates to assess health and disease of animals. We investigated the hematological parameters of indigenous sheep (*Ovis aries*). Blood samples collected from young (group-1), adult male (group-2) and adult female (group-3) sheep were analyzed for total red blood corpuscle (RBC), hemoglobin (Hb) content, packed cell volume (PCV), total white blood cell (WBC), differential leukocytes and erythrocyte sedimentation rate (ESR). Total RBC count was higher in group-3 ($4.81\pm1.98\times10^6$/µL) compared to group-1 ($4.73\pm2.02\times10^6$/µL) and group-2 ($3.12\pm1.22\times10^6$/µL). Hemoglobin content was significantly higher in group-3 (10.70±0.35 g/dL) than group-1 (9.25±0.50 g/dL) ($p<0.05$) and group-2 (9.98±1.79 g/dL). However, PCV was slightly higher in group-1 (35.25±8.06%) and group-3 (35.00±4.83%) than group-2 (32.75±4.65%). By contrast, total WBC count was higher in group-2 ($13.65\pm6.34\times10^3$/µL) than in group-1 ($11.53\pm1.36\times10^3$/µL) and group-3 ($9.49\pm4.08\times10^3$/µL). Differential leukocyte count showed significant decrease of neutrophils in group-2 (17.50±10.08%) compared to group-1 (30.50±3.32%) and group-3 (33.25±8.14%). Although eosinophil count was significantly different among the groups, the number was significantly higher in group-2 (24.00±11.11%) than in group-1 (8.50±2.65%) and group-3 (12.25±2.22%). Lymphocyte (50.25±4.43%-52.00±6.06%), monocyte (4.00±3.65–8.50±2.65%) and basophil (0.25±0.50-0.50±0.58%) count did not differ among the groups. Finally, ESR values were very low (0.32±0.01-0.67±0.01).

Introduction

Sheep are small ruminants having special attributes over other livestock population. There are 3.83 million sheep in Bangladesh (2022-2023) and the population increased gradually to this level from 3.21 million in 2013-14 (DLS, 2022-23). Most of the sheep are indigenous *(Ovis aries*), with few crossbreds and are capable of bi-annual lambing and multiple births (Bhuiyan et al. 2006). They are among the domestic animals which suffer from a number of disease conditions (Gillespie, 2015). Hematological examination provides useful information in assessing the health status and diagnosis of disease of various animal species. The red blood corpuscle (RBC, also known as

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erythrocyte), and white blood cell (WBC, known as leukocyte) are important tools for diagnosis of diseases (Kelly, 2016). Both the physiological and pathological conditions of the animals can be assessed by examination of hematological parameters of blood (Coles, 2014).

A significant increase in erythrocyte number occurs in polycythemia and disturbs tissue fluid balance such as dehydration and other similar derangements of tissue fluid balance giving rise to elevated erythrocyte count. Anemia is a condition that can occur due to an abnormally low number of circulating RBC, abnormally low hemoglobin content in RBC or both (Porth, 2009). The variation in the hemoglobin content could be attributed by differences in age, sex, excitement, pregnancy and diseases affecting heart, lungs and kidneys (Islam et al. 2018; Swenson, 2004). 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) (Bellamy and Olexson, 2000). The information gained from blood parameters would substantiate the physical examination and together with medical history to provide an excellent basis for medical judgment. Hematological parameters have been investigated in sheep in developed countries to improve management practices in enhancing its productivity. Work on hematological parameters in sheep is inadequate in Bangladesh. Therefore, the present study was undertaken to analyze blood for hematological parameters (PCV, ESR, Hb, total RBC, total and differential leucocytes) in indigenous sheep, to compare the parameters with respect to age and sex, and to establish reference indices for comparison of the values in different disease conditions. The research investigated blood parameters of indigenous sheep reared in the Rajshahi city corporation area. Therefore, the finding reflects the real picture of the hematological parameters of sheep native to the area.

Materials and methods

Animal

A total of randomly selected 12 sheep divided into 3 groups designated as group-1 (young) consisting of 4 sheep of <3 months old, group-2 (adult male) consisting of 4 male sheep of >1 year old and group-3 (adult female) consisting of 4 female sheep of >1 year old were used for the experiment for blood parameters. The sheep were reared in a semi-intensive backyard rearing system in the Narikelbaria sheep farm of the University of Rajshahi. The sheep examined were in medium to good body conditions with shiny fleece. The sexes of sheep were determined by visual inspection of external genitalia and age by the physical examination of teeth and matching with dental chart (Ridler and West, 2010). The research was conducted in accordance with the ethical guidelines of the University of Rajshahi.

Collection of samples

Blood samples, 5-7 mL/sheep, collected from jugular vein were placed in test tubes and EDTA (10% solution in distilled water) was added @10-15 µL per mL of blood as an anticoagulant. Smears were prepared on glass slides with fresh/EDTA containing blood and air-dried (Coles, 2014). The blood smears were fixed in absolute methanol immediately after drying and brought to the Laboratory of Veterinary Pathology for further analyses (Relich et al. 2020).

Total erythrocyte count (TEC)

Total erythrocytes were counted on Neubauer's slide. Briefly, the bood was aspirated by RBC pipette up to 0.50 mark and hayem's solution was filled till 101 mark. Four to five drops of mixed sample were discarded and then one drop was added to the RBC counting chamber. The number of erythrocytes was counted in 4 corner and center squares; the results were calculated by multiplying the erythrocyte number with dilution factor (200). The results were expressed as million of RBCs per microliter of blood.

Total leukocyte count (TLC)

Total leukocytes were counted on Neubauer's slide. Briefly,-bood was aspirated up to 0.50 mark in Thoma white pipette and 1% HCl was filled till 11 mark. The sample was allowed to mix for 3 minutes. Four to five drops of mixed sample were discarded and then one drop was added to the white blood cell counting chamber; the cells were counted in 4 corner squares. The results were calculated by multiplying the white blood cell number with dilution factor (20). The results were expressed as thousand of white blood cells per microliter of blood.

Hemoglobin (Hb) content

Hemoglobin content of blood was estimated by following the Sahli's method. Briefly, decinormal hydrochloric acid (0.1N HCl) was taken in the graduated tube up to 2 mark. Thereafter, 20 μLof the anticoagulated blood was aspirated by Sahli's pipette and added in the tube, mixed well by gentle shaking and allowed to stand for 10 minutes. Distilled water was added drop by drop while shaking with a glass stirrer until the color of the reaction product matched with the reference-colored strip of the hemoglobinometer. The result was expressed as gm of hemoglobin per deciliter of blood.

Packed cell volume (PCV)

Wintrobe hematocrit tubes were filled with well-mixed anticoagulated blood up to 10 mark, in duplicate, with a Pasteur pipette and were centrifuged @3000 rpm for 15 minutes. The results were taken as the average volume of two tubes and were expressed as percentage of the volume of erythrocytes in 100 mL of blood.

Erythrocyte sedimentation rate (ESR)

Wintrobe hematocrit tube was filled with anticoagulated blood up to the zero mark of the tube with the help of a Pasteur pipette. The tubes were then placed on an ESR rack at vertical position and kept undisturbed for 60 minutes. The reading was taken as number of millimiter in first hour.

Differential leukocyte count (DLC)

Blood smears were stained with Wright's and Giemsa stains following standard procedure (Jalali et al. 2013). Briefly, methanol-fixed blood smears were incubated with Wright's or Giemsa stain for 5 minutes followed by the addition of equal volume of distilled water for 5 minutes. The smears were then washed with running tap water, air dried and observed under oil objective (100X) using immersion oil. One hundred cells of leukocyte subpopulations such as neutrophil, eosinophil, basophil, lymphocyte and monocytes were counted by meander/battlement methods which involved examination of 3 fields along the margin, 3 fields inward of the margin, 3 fields parallel to the margin, and back 3 fields towards the margin and the procedure was repeated until the cell number reached to 100. The results were expressed as percent of relative number of each leukocyte subpopulations present in the blood.

Statistical analysis

Data are expressed as mean±SD (standard deviation). Statistical analysis was performed by using student's *t*-test and a $p<0.05$ was considered significant.

Results

This study aimed to characterize routine hematological parameters of sheep blood collected from Rajshahi University sheep farm. For this, a total of 12 sheep from three groups designated as group-1 (young), group-2 (adult male), and group-3 (adult female) of Narikelbaria sheep farm of Rajshahi University were examined. Total RBC, total WBC, hemoglobin content, packed cell volume, erythrocyte sedimentation rate, and differential leukocyte counts were performed.

Total Erythrocyte Count (TEC)

Total RBC counts were $4.73\pm2.02\times10^6$/μL, $3.12\pm1.22\times10^6$/μL and $4.80\pm1.98\times10^6$/μL in group-1, group-2 and group-3, respectively (Fig. 1A). Total RBC count did not show any significant difference among the groups. However, the count was higher in group-3 ($4.80\pm1.98\times10^6$/μL) compared to group-1 ($4.73\pm2.02\times10^6$/μL) and group-2 ($3.12\pm1.22\times10^6$/μL).

Total leukocyte count (TLC)

Total WBC counts were $11.53\pm1.36\times10^3$/μL, $13.65\pm6.34\times10^3$/μL and $9.49\pm4.08\times10^3$/μL in group-1, group-2 and group-3, respectively (Fig. 1B). Total WBC count was a slightly higher in group-2 ($13.65\pm6.34\times10^3$/μL) than that in group-1 ($11.53\pm1.36\times10^3$/μL) and group-3 ($9.49\pm4.08\times10^3$/μL).

Hemoglobin (Hb) Content

The hemoglobin contents were 9.25±0.50 g/dL, 9.98±1.79 g/dL and 10.70±0.35 g/dL in group-1, group-2 and group-3, respectively (Fig. 1C). Hemoglobin content was higher in group-3 (10.70±0.35 g/dL) compared to group-1 (9.25±0.50 g/dL) (*$p<0.05$) and group-2 (9.98±1.79 g/dL).

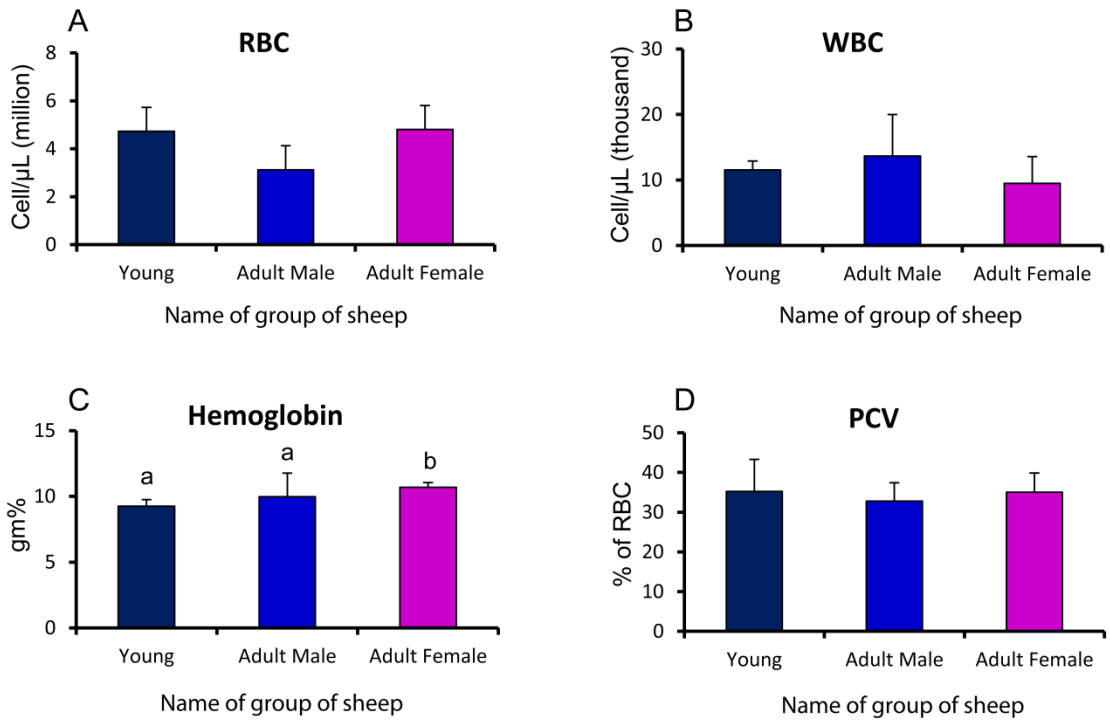

Fig. 1. Total erythrocyte count (A), total leukocyte count (B), Hemoglobin content (C) and Packed cell volume (D).

Packed Cell Volume PCV (%)

The PCV was 35.25±8.06% in group-1, 32.75±4.65% in group-2 and 35.00±4.83% in group-3 (Fig. 1D). Although the PCV was slightly higher in group-1 (35.25±8.06%) and group-3 (35.00±4.83%) than that of group-2 (32.75±4.65%), they did not show any significant difference.

Erythrocyte Sedimentation Rate (ESR)

The ESR values were 0.32±0.01, 0.67±0.01 and 0.33±0.01 in group-1, group-2 and group-3, respectively for the first hour. The ESR value was higher in group-2 than those in group-1 and in group-3 ($p<0.05$), however, the values did not differ significantly between group-1 and group-3.

Differential Leukocyte Count (DLC)

Count of leukocyte sub-population in group-1, 2 and 3 are presented in Fig. 2. Out of the three groups, in group-1, the count of neutrophils, eosinophils, basophils, lymphocytes and monocytes were 30.50±3.32%, 8.50±2.65%, 0.50±0.58%, 52.00±6.06% and 8.50±2.65%, respectively. In group-2, the count of neutrophils, eosinophils, basophils, lymphocytes and monocytes were 17.50±10.08%, 24.00±11.11%, 0.5±0.58%, 50.75±3.30% and 7.25±4.03%, respectively. On the other hand, the count of neutrophils, eosinophils, basophils, lymphocytes and monocytes were 33.25±8.14%, 12.25±2.22%, 0.25±0.50%, 50.25±4.43% and 4.00±3.65%, respectively, in group-3. Differential WBC count showed increased neutrophil count in group-1 (30.50±3.32%) and group-3 (33.25±8.14%) than in group-2 (17.50±10.08%). However, the number did not differ significantly between group-1 and group-2, group-2 and group-3 or group-1 and group-3. Although eosinophil count was higher in group-2 (24±11.11%), however, the number was not significantly different with respect to the number in group-1 (8.50±2.65%) and group-3 (12.25±2.22%). The eosinophil count also did not differ between group-1 (8.50±2.65%) and group-3 (12.25±2.22%). Lymphocyte (50.25±4.43%-52.00±6.06%), monocyte (4.00±3.65–8.50±2.65%) and basophil (0.25±0.50–0.50±58%) counts were not different among the groups.

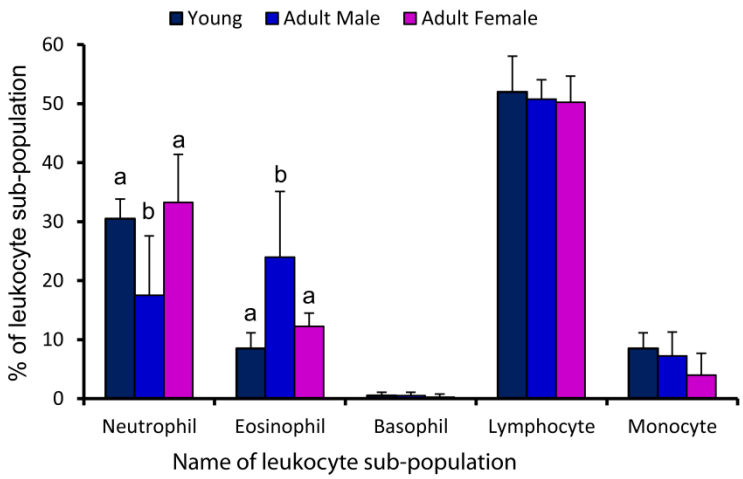

Fig. 2. Percent of leukocyte sub-population in different sheep groups. Note the significant decrease of neutrophil and increase of eosinophil in group-2 (adult male) sheep.

Microscopic examination of leukocyte subpopulation

The subpopulation of leukocytes were recognized by their staining characteristics of nucleus and cytoplasm with dye and morphology of nucleus. Neutrophils were identified by having neutral color cytoplasm with fine granules and dark purple nucleus with 3-5 lobules (Fig.3A). Band neutrophils were recognized by neutral color cytoplasm, and dark purple-blue nucleus with indistinct lobulation (Fig.3B). Eosinophils were detected by full of orange-red coarse granules in the cytoplasm and blue nucleus like a pair of glass with 2-3 lobules (Fig. 3C). The basophils were the largest of the WBCs, spherical in shape with two-lobed black nucleus containing tiny purple polka dot granules. On the other hand, the monocytes had abundant sky blue cytoplasm and purple nucleus of large kidney shape or with slight indentation (Fig. 3C). The lymphocytes possessed clear, pale blue scant cytoplasm with purple red round nucleus (Fig. 3D). The lymphocytes were the smallest WBC.

Discussion

Research on hematological paramaters in sheep is very rare in Bangladesh. Considering this view, this research attempted to determine the different hematological parameters of apparently healthy indigenous sheep to develop a baseline data and to aid in the diagnosis of disease conditions. The values of different hematological parameters were examined in apparently healthy 3 groups of sheep including young, adult male and adult female and the results were compared among them.

Total erythrocyte count (TEC)

In the present study, Total Erythrocyte Count (TEC) in apparently healthy sheep was lower in adult males than in young and adult females (Fig: 1A). Generally, the TEC values are higher in male sheep than female ones (Olayemi et al. 2000). On the other hand, in female sheep, TEC values are lower due to the activity of estrogen hormone on the erythropoietic processes which leads to relative decrease of RBC production (Coles, 2014). Other factors responsible for the variation might be due to environment, breed, feeding, management and altitude (Egbe-Nwiyi et al. 2000). Works on hematological profile of fat-tailed sheep reported similar TEC count in juvenile, adult male and adult females in agreement with the notion (Islam et al. 2018). The findings of decreased TEC count in adult male may be attributed by dehydration or gastrointestinal parasitism. Gastrointestinal parasites, especially stomach worms, are known to cause loss of erythrocytes in domestic sheep (Flay et al. 2022). As gastrointestinal parasitic infection is the most important limiting factor of sheep productivity, parasitism has a highly detrimental effect on the sheep industry (Jones, 2001).

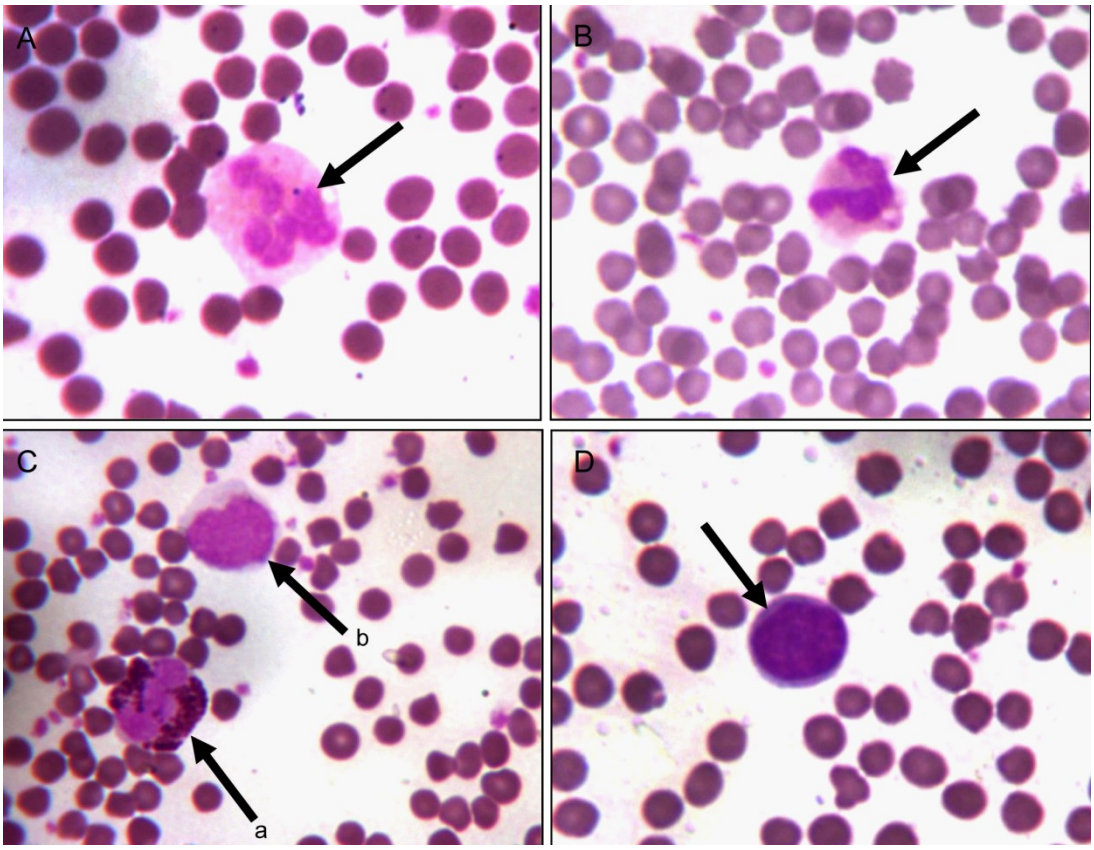

Fig. 3. Representative images of mature neutrophil (A, arrow), immature/band neutrophil (B, arrow), eosinophil (C, arrow-a), monocyte (C, arrow-b), and lymphocyte (D, arrow).

Total leukocyte count (TLC):

In the current study, the adult male sheep (group-2) showed increment of WBC count compared to young (group-1) and adult female (group-3) groups although values did not reach significant level (Fig: 1B). The increased WBC count in the adult male sheep may be due to the immune response of body against the parasitic infection as a means of self-defense or it may be due to increased sensitivity to the protein of the parasite, which is foreign to the animal's body (Bakker et al. 2004). Increased total WBC count may also indicate tissue damage and resultant concurrent bacterial infection. However, the total WBC count excluded the possibility of tissue damage related to bacterial infection indicated by an absence of increment of body temperature or neutrophil count. The higher values of WBC observed may also be attributed to the extensively managed sheep which make them face challenges from microbes when graze on free range. Some study demonstrated increased total WBC count in adult male sheep over the adult females (Islam et al. 2018).

Hemoglobin (Hb) content

The hemoglobin concentration in young sheep (group-1) was lower than those in adult male and female sheep and these findings are in agreement with the notion that the blood parameters increase with aging (Islam et al. 2018). However, several factors including animal strains, gender, geographical distribution, parasitic infection and health conditions affect the hemoglobin content (Bhat et al. 2011).

Packed cell volume (PCV)

The PCV values in young sheep showed significant increment compared to adult female sheep although there was no significant difference with adult male sheep or between adult male and adult female sheep. Studies conducted in other laboratories on sheep also demonstrated significantly increased PCV in juvenile sheep

compared to in adult sheep (Islam et al. 2018; Rahman et al. 2018). Apparently, the increased PCV results in increased hemoglobin count in polycythemia. However, the PCV is affected by the maturation of the erythrocytes, the immature erythrocytes are responsible for higher PCV. The increased rate of erythropoiesis due to increased rate of metabolism in young sheep may have contributed to the increased PCV (Rahman et al. 2018). Stomach worm infection in small ruminants is an important cause of chronic blood loss and resultant decrease of PCV and development of anemia (Mir et al. 2007; Sharma et al. 2000). However, the sheep under investigation were apparently healthy and demonstrated PCV values within the normal ranges.

Erythrocyte sedimentation rate (ESR)

The mean values of ESR in apparently healthy sheep were very low (0.32±0.01-0.67±0.01 mm/hr) and did not show significant differences between the young and adult females although the values were higher in adult males ($p<0.05$), these findings are in agreement with the findings of Rahman et al. (2018), who reported ESR below 1.00 in juvenile and adult sheep. One reason of low ESR values may be that the sheep examined were healthy and the high content of plasma protein in sheep (Gupta et al. 2003). Inflammation causes change in clotting protein concentration (fibrinogen and globulin) and results in erythrocytes to clump together that settle more quickly, therefore, increased ESR in adult male sheep indicate progression of inflammation due to parasitic infection or autoimmune disease (Harrison, 2015).

Differential leukocytes count (DLC)

Neutrophil: Neutrophils are the first line of defense and destroy invading bacteria by phagocytosis (van Kessel et al. 2014; Worku et al. 2021). The present study showed decreased neutrophil count (Fig: 5) in adult male sheep (group-3) in contrast to young (group-1) and adult female (group-3) sheep.

Eosinophil: Eosinophils are considered to be important elements in the response against fungal and parasitic infection (Balic et al. 2002; Terefe et al. 2005). Increased eosinophil affects specific tissues of the body as asthma in lungs and cystitis in bladder (Makol et al., 2022; Nakagome and Nagata, 2023). Eosinophil count (Fig: 5) in adult male sheep (group-2) increased by 3-fold in contrast to young (group-1) and 2-fold in contrast to adult female (group-3) sheep. These findings may indicate that the total WBC count in the adult male sheep was increased due to the increase in the number of eosinophils, an indication of parasitic infection.

Basophil: Basophil count of the study was within normal range. Basophils are one out of the three granulocytes and the smallest in number. Generally, they function to enhance blood flow by releasing an enzyme named histamine and promote healing. Basophils also prevent blood from clotting too quickly by releasing another enzyme called heparin. A decrease in basophil number, basopenia, may be due to the results of basophils working overtime to attack an allergen or treat an infection taking longer than normal to heal or hyperfunction of thyroid (Dale, 2023). By contrast, basophilia, an increase in basophil number may be attributed by decreased production of thyroid hormone or bone marrow disorder mimicking production of white blood cells as in leukemia (Dale, 2023).

Lymphocyte: Lymphocytes are important members of mononuclear phagocytic system and display critical roles in innate and adaptive immunity. B-lymphocytes uptake antigens processed and presented by macrophages, transformed into plasma cells and synthesize humoral antibody that targets bacteria, viruses and other invading microorganisms (Gordon, 2016). Other type of B-lymphocytes transformed into memory cells and contribute in rapid antigen recognition and antibody production. T-lymphocytes, on the other hand, directly attack and kill microorganisms and cancer cells (Gordon, 2016). Lymphocyte counts within the normal range in all the three group of sheep indicated healthy body condition.

Monocytes: Blood monocytes, by changing phenotypes, turn into dendritic cells and macrophages in tissues and serve to alert body about infection, clear necrotic and apoptotic tissue debris, invading microorganisms and play pivotal roles in innate immunity (Parihar et al. 2010). Finally, the study investigated hematological parameters in young, adult male and adult female sheep in absence of overt health problems, however, the findings indicated that adult male sheep (group-2) might have been suffering from parasitic infection manifested by increased ESR,

decreased total RBC and increased total WBC due increment of eosinophils.

Conclusion

Blood is an important element for profiling of pathophysiological conditions in an organism. Important functions of the blood are carried out by the individual and collective actions of its hematological constituents and blood cells. Profiling of hematological parameters (PCV, ESR, Hb, total RBC, total and differential WBCs) are, therefore, widely used for assessment of health status and abnormality both in humans and animals. This work investigated blood parameters of indigenous sheep reared in Rajshahi. Therefore, findings of this work reflect the real picture of the hematological parameters of sheep native to the area and will serve as baseline data to help assess health condition and diagnosis of disease.

Author's contribution

Conceptualization, HMG, SMAR; methodology, MNK, RP, RK, AA, SRK, SMAR, HMG; formal analysis, MNK, RK, AA, SRK; validation, RP, SMAR, HMG; investigation MNK; visualization, MNK, HMG; writing—original draft preparation, MNK; writing—review and editing, MNK, RP, RK, AA, SRK, SMAR, HMG; resources, SRAR, HMG; supervision SMAR, HMG. All authors have read and agreed to the published version of the manuscript.

Conflict Interest: The authors declare no conflicts of interests.

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