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SHORT COMMUNICATION

Cytomorphological Characteristics of Peripheral Blood Cells in Adult Buffalo (*Bubalus bubalis*)

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A B S T R A C T

Cytomorphological studies are vital for detecting blood diseases in buffalo. The size and structure analysis of blood cells identifies abnormalities indicating infections, anemia, leukemia, or parasites like Babesia spp. or Theileria spp. This enables the prompt diagnosis and treatment of such diseases. Cytomorphology-based studies could be intensively helpful in promoting buffalo health and enhancing productivity, besides being cost-effective and minimally invasive. These studies complement diagnostic tools and improve veterinary hematology and disease surveillance, supporting animal health and agricultural sustainability. Hence, the present study was designed to explore the detailed cytomorphological characteristics of blood cells in adult buffalo. Twenty-four blood samples were collected irrespective of sex. Air-dried thin blood smears were prepared and stained with MGG stain to facilitate the analysis of various cellular components present in buffalo blood. The thin buffalo blood smears were examined under a magnification of 1000X utilizing an Olympus CX33RTFS2 microscope. The observed blood cells were RBCs, WBCs, and platelets. WBCs were further categorized into granulocytes and agranulocytes. The erythrocytes of buffalo were found to be non-nucleated and round. Neutrophils appeared as rounded cells with the cytoplasm containing granules. Eosinophils also had a round outline featuring distinct cytoplasmic granules. Basophils were observed infrequently, characterized by distinct blue granules in the cytoplasm. Three types of lymphocytes were observed in the present study: small, medium, and large. Monocytes were noted to be round, while platelets were irregular and round in structure. Occasionally, reticulocytes were observed, appearing as small, thin blue rods or granules. In conclusion, the light microscopic analysis of buffalo blood cells revealed similarities to those of some previously studied animals, with only a few minor morphological differences.

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INTRODUCTION

The blood profile of animals is vital for the confirmation of clinical diagnoses and the estimation of the severity of diseases (Piccione, 2010; Jaramillo *et al.,* 2024). Hence, the buffalo-specific cytomorphological studies are crucial due to the unique hematological characteristics of buffalo compared to other livestock. These animals exhibit distinct cellular morphology and immune responses, which can impact the interpretation of blood smears and disease diagnosis. Generic reference values may result in misdiagnosis or delayed treatment. By concentrating on species-specific cellular features, such as variations in the leukocyte and erythrocyte morphology, veterinarians can diagnose the infectious and parasitic diseases as well as hematological disorders more accurately. These studies are vital for improving disease management, enhancing productivity, and safeguarding the health of buffalo, which play an essential role in dairy and agricultural economies across many regions.

Blood is a specialized fluid connective tissue composed of plasma, a colorless substance, and formed

elements, which include red blood cells (erythrocytes), white blood cells (leukocytes), and platelets (thrombocytes). Various formed elements of blood and platelets are suspended within the plasma, as demonstrated in Fig. 1 (Atkins *et al.*, 2017; Choudhary *et al.*, 2021; Doley *et al.*, 2023, 2024; Aslam *et al*., 2023). The erythrocytes (RBCs) are the most common blood cells, accounting for approximately 94% of the total blood cells, whereas the white blood cells (WBCs) are comparatively less numerous and account for less than 1% of the total blood cells. WBCs contain a nucleus and other organelles but do not contain hemoglobin, unlike RBCs. WBCs are part of the immune system, and their primary function is to provide a defense mechanism against various animal diseases, protecting against the caused by pathogens and removing the damaged cells, toxins, and wastes from the animal body. WBCs are classified into two groups based on the presence or absence of cytoplasmic granules, i.e., granulocytes and agranulocytes. Granulocytes or granular leukocytes have cytoplasmic granules and have been classified into three subtypes: neutrophils, eosinophils, and basophils. Agranulocytes, or agranular leukocytes, show the absence of cytoplasmic granules and have been divided into two subtypes: monocytes and lymphocytes.

Blood cell studies are important from various perspectives, including morpho-physiological, clinicopathological, and therapeutic aspects (Hasan *et al.*, 2023). Blood examinations are crucial in assessing animals' general health and disease diagnosis (Choudhary *et al.*, 2023a). These tests are routinely performed to assess health status, diagnose hematological conditions, determine the body's response to hematological challenges, and monitor the progression of certain diseases (Sarkar *et al.*, 2023). Changes in the blood cell count, the intricate architecture of the cell morphology, and a diverse array of intracellular components can function as vital indicators, providing a clear snapshot of an animal's health status. (Ishikawa *et al.*, 2008; Fang *et al.*, 2014; Wang *et al.*, 2021).

Blood smear examinations are essential for the cytomorphology of blood cells, including RBCs, WBCs, and thrombocytes. The advancement in microscopic techniques and blood cell staining methods became crucial in identifying acute and chronic leukemias, a narrative that spanned the entire 20th century (Gini, 2024). One important aspect observed in blood films is lymphocyte vacuolation, which is clinically significant for patients suspected to be suffering from metabolic diseases (Anderson *et al.*, 2005). Examination of the blood cells is crucial for the diagnostics. An experienced pathologist can determine specific disease conditions or identify underlying causes, which may not be evident to a clinician, simply by analyzing the structure and morphological changes (Banga *et al.*, 2020).

Cytomorphology-based studies could be crucial in enhancing and promoting the health of buffaloes. These are cost-effective, minimally invasive, and complement other diagnostic tools, thereby enhancing veterinary hematology and disease surveillance for better animal health and agricultural sustainability.

Artificial Intelligence has significantly transformed the veterinary anatomical and morphological sciences by facilitating advancements in the diagnostics, research methodologies, and treatment strategies (Anwar *et al.*, 2023; Choudhary *et al.*, 2023, 2025; Dablool *et al.*, 2024; Choudhary, 2025; Vickram *et al.*, 2025). Many scientists have utilized AI tools to demonstrate various blood cells, as the application of AI for the cytomorphological classification of blood cells minimizes interobserver variability and subjective human error (Hu *et al.*, 2022; Ilić *et al.*, 2023; Xing *et al*., 2023; Campos-Medina *et al.*, 2024); however, the need of traditional methods for the preparation of blood films in cytomorphology remains unchanged.

Previous studies have documented blood cell morphology in buffalo calf, goat, donkey, cattle, and pig (Singh, 2000; Menaka, 2003; Menaka and Singh, 2006; Mehta, 2010; Mrigesh, 2011; Sarkar *et al.*, 2022; Choudhary *et al.*, 2023b). The existing scientific literature on the cytomorphology of blood cells in buffalo is notably limited in detail. Keeping this in mind, the present investigation aims to conduct a comprehensive cytomorphological study of the blood cells in the buffalo.

MATERIALS AND METHODS

A total of twenty-four blood samples from adult buffaloes, regardless of sex, were collected to evaluate blood cells' cytomorphology from January to April 2025. The peripheral blood samples (2mL) were received from the Teaching Veterinary Clinical Complex (TVCC), College of Veterinary Science, Rampura Phul, Punjab, in sterile and siliconized tubes filled with Ethylenediaminetetraacetic acid (EDTA) to avoid blood coagulation. Institutional animal ethical approval was not required for this study, as the samples were obtained from the TVCC of the college.

The thin blood smears were prepared immediately on the clean, dry, and grease-free slides for cytomorphological studies. The blood smears were air-dried and stained with the May-Grünwald Giemsa (MGG) stain (Bover, 1964) for differential staining of blood cells. The stained slides with MGG were visualized at 1000X magnification under an Olympus CX33RTFS2 microscope (Japan), and different blood cell images were captured with an Olympus camera attached to the microscope (Japan) for illustrative purposes. The magnification used for capturing the buffalo blood cells was 1000X. The captured images were labeled appropriately using Adobe Photoshop version 24.0 and Microsoft Publisher (Microsoft 365). Cytomorphological data of buffalo blood cells were analyzed statistically (Snedecor and Cochran, 1994). The prepared blood slides were stored in slide boxes and used to teach the light microscopic morphology of blood cells to the first-year veterinary students at the college.

RESULTS

Blood smears stained with MGG stain were meticulously analyzed under a light microscope using oil immersion at a magnification of 1000X. Various buffalo blood cells examined in this cytomorphological study have been illustrated in Fig. 2A-D and 3A-D. The cytomorphometric data of the blood cells of buffalo have been presented in Table 1 as mean±SE, and a comparison with some other animals, such as cattle, pig, mule, sheep, and goat blood cells, has also been given as per the available scientific literature.

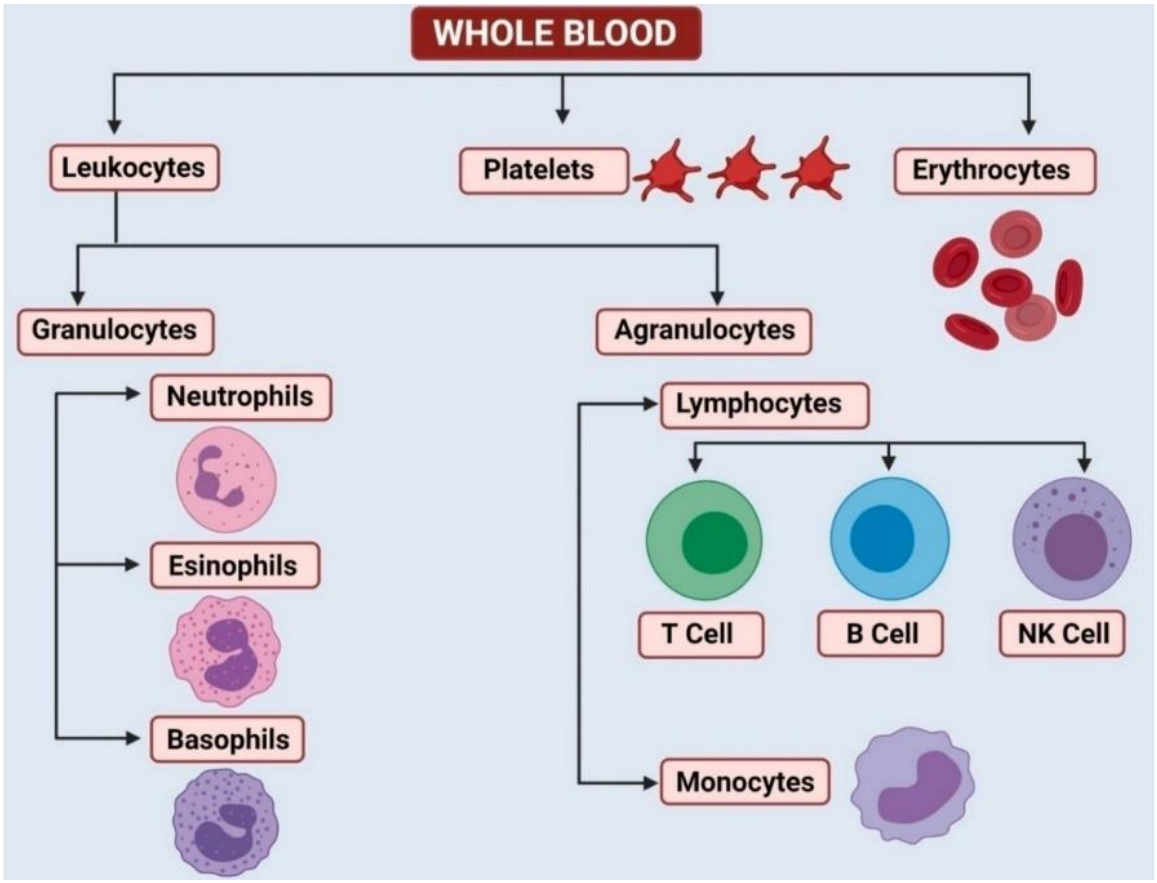

Fig. 1: Diagrammatic representation of the various blood cells of buffalo. Reproduced from Choudhary *et al.* (2021) under Creative Commons Attribution 4.0 International License (**https://creativecommons.org/licenses/by/4.0/**).

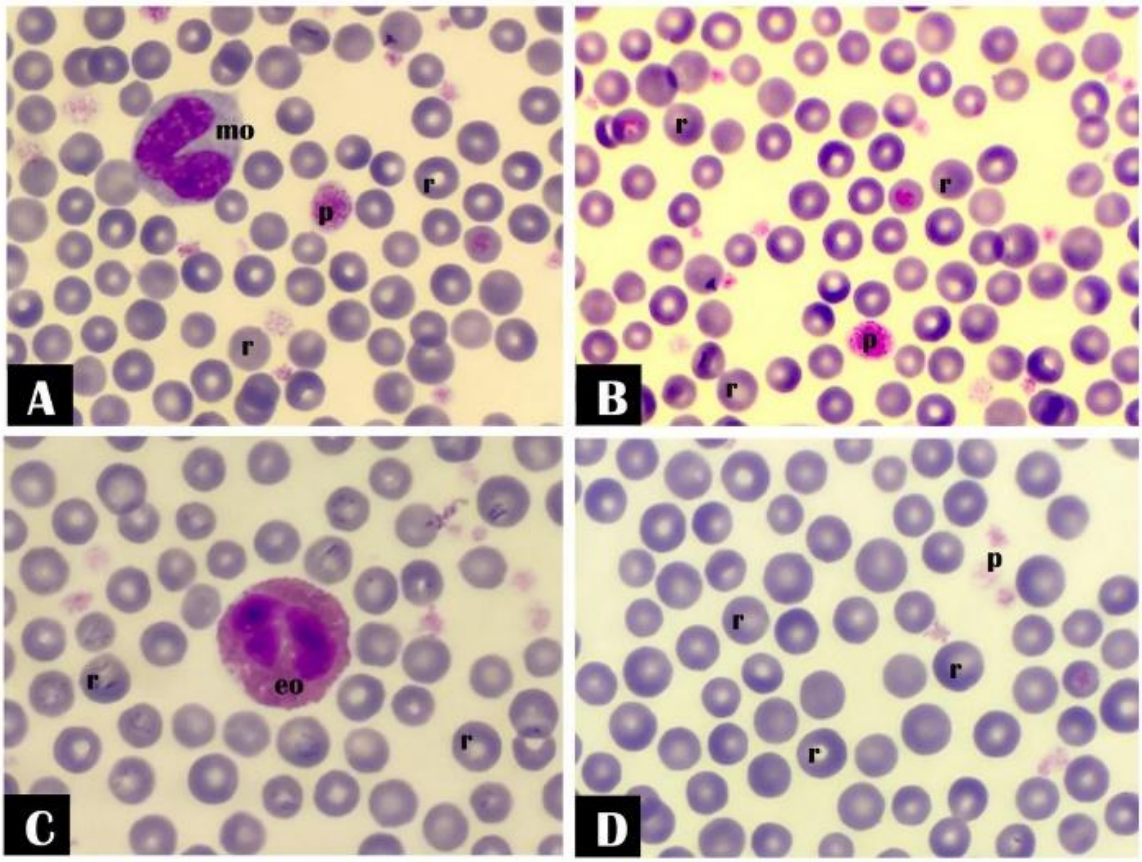

Fig. 2: Microstructure of peripheral blood cells of buffalo (MGG staining) A: showing erythrocytes, monocyte, platelet (r,mo,p); B: erythrocytes, platelet (e,p); C: erythrocytes, eosinophil (r,eo) and D: erythrocytes, platelet (e,p). The magnification was 1000X.

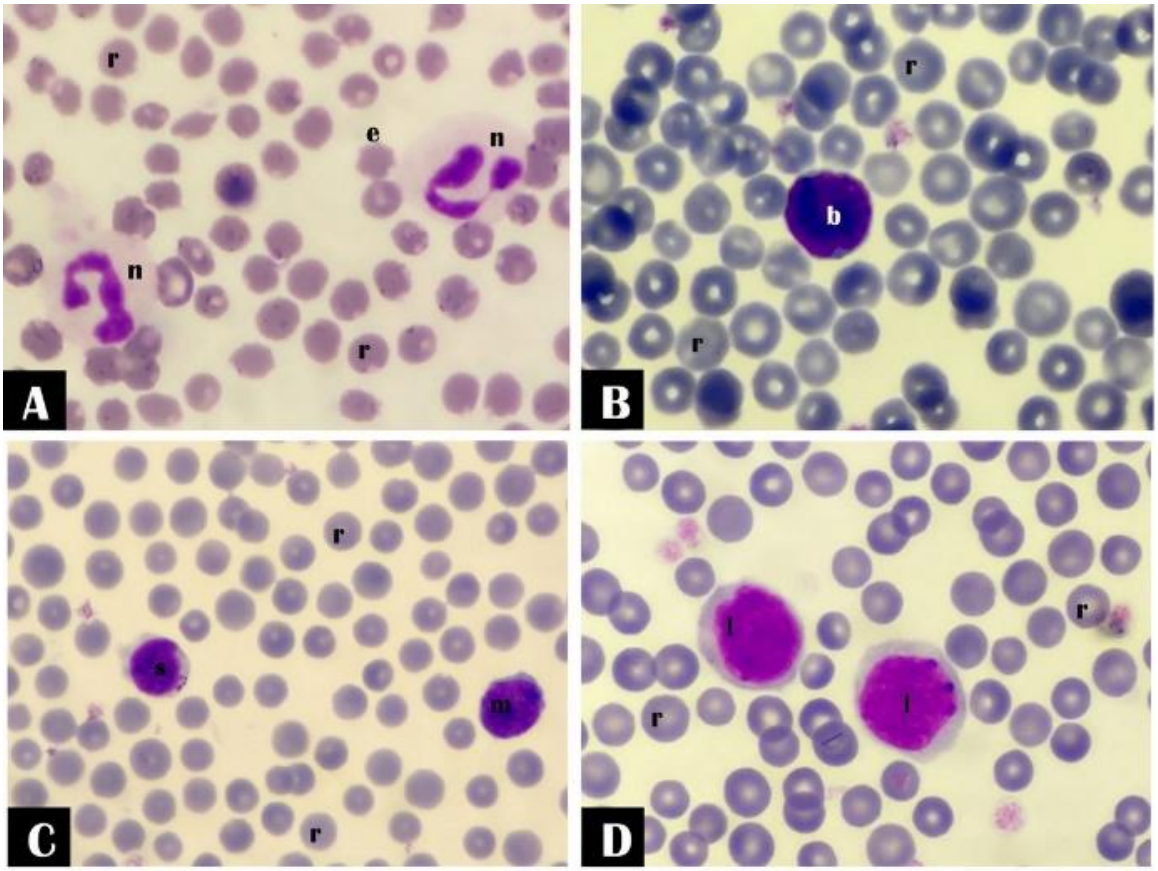

Fig. 3: Microstructure of peripheral blood cells of buffalo (MGG staining) A: showing erythrocytes, echinocyte, neutrophils (r,e,n); B: erythrocytes, basophil (e,b); C: erythrocytes, small lymphocyte, medium lymphocyte (r,s,m) and D: erythrocytes, large lymphocytes (e,p). The magnification was 1000X.

Table 1: Cytomorphometric comparison of the various blood cells of buffalo with other species based on available literature.

Blood cells		Buffalo	Zobawng cattle	Pig	Mule	Sheep	Goat
Erythrocyte		5.02±0.17µm	6.42±0.13µm	5.70±0.17µm	6.25±0.15µm	4.10±0.27µm	3.78±0.47µm
Neutrophil		16.52±0.23µm	11.21±0.28µm	12.00±0.44µm	12.25±0.27µm	11.20±0.24µm	10.16±0.76µm
Eosinophil		15.51±0.23µm	13.42±0.27µm	12.25±0.28µm	13.40±0.24µm	14.50±0.26µm	13.60±1.25µm
Basophil		11.54±0.23µm	12.42±0.38µm	13.25±0.24µm	13.80±0.27µm	13.60±0.14µm	13.05±2.90µm
Lymphocyte	Small	11.17±0.15µm	8.05±0.13µm	7.44±0.23µm	5.95±0.15µm	8.60±0.27µm	10.11±1.12µm
	Medium	13.50±0.08µm	9.08±0.12µm	9.65±0.16µm	6.85±0.19µm	11.80±0.10µm	11.12±0.93µm
	Large	16.83±0.13µm	11.83±0.17µm	12.05±0.23µm	9.40±0.19µm	14.90±0.27µm	14.95±1.71µm
Monocyte		13.48±0.10µm	13.54±0.18µm	13.20±0.27µm	13.40±0.19µm	16.20±0.29µm	12.49±1.18µm
Platelet		3.41±0.26µm	1.83±0.21 µm	2.76±0.33µm	1.75±0.2µm	NR	0.5-1.0±0.31µm
Reference		Present Study	Sarkar *et al.* (2022)	Mehta and Singh (2014); Mehta *et al.* (2013)	Mrigesh *et al.* (2016)	Kumar *et al.* (2010)	Menaka and Singh (2006)

Note: NR stands for not reported in the study.

Buffalo peripheral blood cells were classified into RBCs, WBCs, and thrombocytes (platelets) based on their morphology, size, nucleus characteristics, and cytoplasmic features (Fig. 1). The buffalo RBCs were found to be non-nucleated and round. The current study revealed the presence of a few numbers of biconcave and crenated erythrocytes, commonly referred to as echinocytes.

The buffalo blood granulocytes in this study consisted of neutrophils, eosinophils, and basophils. Neutrophils appeared round, with the cytoplasm containing granules (Fig. 2A-D and 3A-D). The diameter of the erythrocytes was 5.02±0.17µm in the present study. The neutrophils were roughly spherical, with two to four-lobed nuclei having different shapes, sizes, and numbers, including alphabets C, O, S, U, and Z (Fig. 3A). The diameter of the neutrophils was 16.52±0.23µm. Eosinophils also had a round outline featuring distinct cytoplasmic granules with 15.51±0.23µm diameter (Fig. 2C). Basophils were reported to be infrequently characterized by distinct blue cytoplasmic granules (Fig. 3B). The basophil diameter was 11.54±0.23µm.

The buffalo blood agranulocytes in this study consisted of monocytes and lymphocytes. The monocytes were round with bilobed nuclei and measured 13.48±0.10µm in diameter (Fig. 2A). Between the erythrocytes, the platelets were irregular to rounded, reddish-pink cells. The size of the platelets ranged between 2 to 7µm; however, the mean diameter of the platelets was measured to be 3.41±0.26µm. Three types of lymphocytes were found in the present study: small, medium, and large (Fig. 3C, D). The diameters of small, medium, and large lymphocytes were found to be 11.17±0.15, 13.50±0.08, and 16.83±0.13µm, respectively, in the present study. Occasionally, reticulocytes were reported in buffalo blood, appearing as small, thin blue rods or

granules. In conclusion, the light microscopic analysis of buffalo blood cells revealed similarities to those of other previously studied animals, with only a few minor morphological differences.

DISCUSSION

The study classified the buffalo peripheral blood cells into RBCs, WBCs, and thrombocytes (platelets) based on their morphology, size, nucleus characteristics, and cytoplasmic features. In the present study, buffalo RBCs were circular and non-nucleated with a 5.02±0.17µm diameter. The peripheral area of RBCs stained darker in ring form, and the middle area was stained pale with the May-Grünwald Giemsa (MGG) stain, as mentioned earlier in buffalo calf (Singh, 2000), pig (Mehta, 2010), donkey (Mrigesh, 2011), mule (Mrigesh *et al.*, 2016), Zobawng cattle (Sarkar *et al.*, 2022), and indigenous (Zovawk) pig of Mizoram (Choudhary *et al.*, 2023b). A few erythrocytes displayed a deep, small, rounded area that appeared pale-stained, as described earlier in goat (Menaka and Singh, 2006) and Zobawng cattle (Sarkar *et al.*, 2022). In the present study, some larger erythrocytes with a diameter of 6-7µm were also observed, similar to those of donkey (Mrigesh, 2011), Zobawng cattle (Sarkar *et al.*, 2022) and indigenous (Zovawk) pig of Mizoram (Choudhary *et al.*, 2023b). The current study revealed the presence of a few biconcave and crenated erythrocytes, commonly referred to as echinocytes, as also reported in the cattle (Parveen *et al.*, 2023).

The neutrophils were almost spherical cells with a 16.52±0.23µm diameter. The nuclei exhibited two to four lobes with varying shapes, sizes, and quantities. The lobes were observed in diverse forms, including C, O, S, U, and Z, as mentioned previously in buffalo calf (Singh, 2000), donkey (Mrigesh, 2011), mule (Mrigesh *et al.*, 2016), Zobawng cattle (Sarkar *et al.*, 2022) and indigenous (Zovawk) pig of Mizoram (Choudhary *et al.*, 2023b). Chromatin material displayed light and dark areas within the nuclear lobes of neutrophils. The dark-stained regions were typically located on the periphery, while the light-stained areas were found in the center of the neutrophil, as also reported in donkey (Mrigesh, 2011), Zobawng cattle (Sarkar *et al.*, 2022), and indigenous (Zovawk) pig of Mizoram (Choudhary *et al.*, 2023b). The neutrophil cytoplasm was observed to be pale-pinkish blue, as reported in donkey (Mrigesh, 2011), sheep (Kumar *et al.*, 2010), Zobawng cattle (Sarkar *et al.*, 2022) and indigenous (Zovawk) pig of Mizoram (Choudhary *et al.*, 2023b).

The eosinophils were approximately circular cells with 15.51±0.23µm diameter featuring two to three nuclear lobes. Thick chromatin strands linked the lobes, as reported earlier in buffalo calf (Singh, 2000), goat (Menaka and Singh, 2006), sheep (Kumar *et al.*, 2010), Zobawng cattle (Sarkar *et al.*, 2022), and the indigenous (Zovawk) pig of Mizoram (Choudhary *et al.*, 2023b). In the present study, dark-stained regions of chromatin material were predominantly identified at the periphery. The cytoplasm displayed an eosinophilic coloration and was characterized by the presence of cytoplasmic granules, similar to those observed in donkey (Mrigesh, 2011), Zobawng cattle (Sarkar *et al.*, 2022) and indigenous (Zovawk) pig of Mizoram (Choudhary *et al.*, 2023b). The large, round cytoplasmic granules exhibited a pronounced eosinophilic coloration. These granules were densely packed and uniformly distributed throughout the cytoplasm, highlighting their structural organization as reported earlier in Zobawng cattle (Sarkar *et al.*, 2022) and the indigenous (Zovawk) pig of Mizoram (Choudhary *et al.*, 2023b).

Basophils were spherical with a diameter of 11.54±0.23µm. Two to three lobes were eccentrically arranged in the basophil nuclei as reported in buffalo calf (Singh, 2000), goat (Menaka and Singh, 2006), sheep (Kumar *et al.*, 2010), and Zobawng cattle (Sarkar *et al.*, 2022). The cytoplasm displayed numerous granules of varying sizes, characterized by violet and pink hues that filled the entire cytoplasmic region. The larger violet granules exhibited a deeper staining, whereas the lighter-stained granules were comparatively larger than the eosinophils. Singh (2000) identified the presence of large basophil granules within the cell matrix of blood samples obtained from a buffalo calf.

The lymphocytes varied in size, ranging from small to large, as reported in donkey (Mrigesh, 2011), Zobawng cattle (Sarkar *et al.*, 2022), and the indigenous (Zovawk) pig of Mizoram (Choudhary *et al.*, 2023b). In the current study, small lymphocytes were frequently recorded, with a diameter of 11.17±0.15µm. A thin ring of pale cytoplasm encircled the spherical nucleus. The nuclear chromatin of small lymphocytes was stained darkly, as reported in Zobawng cattle (Sarkar *et al.*, 2022) and the indigenous (Zovawk) pig of Mizoram (Choudhary *et al.*, 2023b). The medium lymphocytes were determined to have a diameter of 13.50±0.08µm. Observations revealed the presence of distinctly dark-stained regions alongside lighter-stained patches within the nuclear chromatin. Medium lymphocytes contained more cytoplasm than small and large lymphocytes, as reported in buffalo calf (Singh, 2000), goat (Menaka and Singh, 2006), sheep (Kumar *et al.*, 2010), Zobawng cattle (Sarkar *et al.*, 2022), and indigenous (Zovawk) pig of Mizoram (Choudhary *et al.*, 2023b). The large lymphocytes were present in smaller numbers, measuring an average diameter of 16.83±0.13µm. Their nuclei were eccentrically positioned, and the nuclear chromatin displayed a relatively higher number of faintly stained patches. The cytoplasm was abundant and stained a light bluish color when treated with MGG stain, as reported in sheep (Kumar *et al.*, 2010), Zobawng cattle (Sarkar *et al.*, 2022), and the indigenous (Zovawk) pig of Mizoram (Choudhary *et al.*, 2023b).

The monocytes were round, featuring bilobed nuclei, and had a diameter measuring 13.48±0.10 µm. The nucleus was indented and positioned eccentrically in most of the cells, as also reported in equine (Grondin and Dewitt, 2010), donkey (Mrigesh, 2011), Zobawng cattle (Sarkar *et al.*, 2022), and the indigenous (Zovawk) pig of Mizoram (Choudhary *et al.*, 2023b). In the current study, vacuoles were shown within the cytoplasm, creating a foamy appearance when stained with MGG, consistent with the findings in buffalo calf (Singh, 2000), goat (Menaka and Singh, 2006), and Zobawng cattle (Sarkar *et al.*, 2022).

The platelets were observed as irregular to rounded, reddish-pink cells between the erythrocytes, as reported earlier in sheep (Kumar *et al.*, 2010), Zobawng cattle (Sarkar *et al.*, 2022), and the indigenous (Zovawk) pig of

Mizoram (Choudhary *et al.*, 2023b). The size of the platelets ranged between 2 to 7µm; however, the mean diameter of the platelets was measured to be 3.41±0.26µm.

The reticulocyte count is monitored intermittently, as these cells form approximately one percent of the total blood volume. They were spherical in shape and displayed a distinct pattern of reticulin filament arrangement similar to that observed in donkey (Mrigesh, 2011), Zobawng cattle (Sarkar *et al.*, 2022), and the indigenous (Zovawk) pig of Mizoram (Choudhary *et al.*, 2023b). The reticulofilamentous material was effectively stained blue in the present study, revealing its presence as small, thin rods or granules. This material prominently formed a distinct ring around the periphery of the cell, demonstrating a clear and consistent distribution pattern. The reticulofilamentous material was evenly distributed throughout the cytoplasm. A few reticulocytes were also reported in goat (Menaka and Singh, 2006), sheep (Kumar *et al.*, 2010), Zobawng cattle (Sarkar *et al.*, 2022), and indigenous (Zovawk) pig of Mizoram (Choudhary *et al.*, 2023b).

A key limitation of this study was the lack of systematic clinical and laboratory evaluations of the animals before sample collection. Nonetheless, we established the absence of disease through thorough history taking and physical examinations, ensuring that only healthy animals were selected for the study.

Conclusions: In conclusion, the blood cells of buffalo exhibit similarities to those of other domestic animals, with only minor cytomorphological differences as compared to the cattle, goat, horse, pig, and buffalo calf. This research decisively establishes baseline data on all blood cell types in buffalo, providing a critical foundation for future investigations in cytomorphology. The study of the cytomorphology of buffalo blood cells is crucial in veterinary diagnostics, as it aids in identifying infections, anemia, and various hematological disorders. This examination enables veterinarians to evaluate immune responses and monitor disease progression. In an educational context, it enhances students' understanding of the normal and abnormal blood cell structures, fostering practical skills in hematology and comparative animal pathology.

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Authors contribution: OPC conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or table, and approved the final draft. JS performed the experiments, analyzed the data, prepared figures and/or table, and approved the final draft. CM collected samples and analyzed the data, reviewed drafts of the paper, and approved the final draft. PC conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft. All authors interpreted the data, critically revised the manuscript for important intellectual content, and approved the final version.

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