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Morphological and morphometrical analyses of Blood Cells of Emus (*Dromaius novaehollandiae)* in Captive condition

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Abstract

The designed of the study was made for analyses of morphology and morphometry (i.e., measurement) of cells of blood of emus in captive condition. Since studies on morphological features of erythrocytes and leucocytes of captive emus were not properly documented in literature, to know the various shapes of erythrocytes (RBCs) and leucocytes of them and to study morphometry of blood cells in four different months this work is undertaken. For observance of cellular morphology of erythrocytes and leukocytes in them total fourteen emus were taken for the present study. From the jugular veins sample blood were taken by an expert from one private farm of Bhubaneswar, Odisha, India and transferred into EDTA vials and blood smears were made on clean microscopic slides. Then staining of blood smears were done with Giemsa stain to study the cellular morphology. The findings of the study reveal that captive emus have various shapes of erythrocytes and by this type of study shapes of normal erythrocyte and abnormal erythrocytes can be known. RBCs and their nuclei and leucocytes were photomicrographed and observed below the microscope under 40X objective. Length and breadth of RBCs and their nuclei, lymphocytes and their nuclei and other leucocytes were measured with the help of ocular micrometer and stage micrometer. Mean and standard error were calculated for statistical analyses.

Keywords: Emu, RBCs, lymphocyte, monocyte, heterophil, eosinophil, basophil.

Introduction

Being native to Australia, the living bird of the world emu (Figure-1) is the second largest bird by height (first being ostrich) belonging to the order Ratite and is an important cultural icon of Australia. The large and powerful birds having legs which are strongest, powerful in nature are emus which have defensive nature for their young. The plumage of female darkens slightly whereas plumage of male remains unchanged although there are some colour changes below the eyes both in male and female. During mating season the tracheal pouch of them are more prominent. Farming of it is now in different parts of India and Odisha privately because of demand for consumption and profitability. Its farming is very convenient owing to its hardy nature, better survival and cost effective feeding. Now a days in the business sector of SA and Australia breeding of emus is a million-dollar business and is a worldwide business now. By processing the abdominal fat of them oil is processed for therapeutic uses. Long before the arrival of British ships on the east coast of the country, the fat of emu is used by the native inhabitants of Australia to control the pain. The abdominal fat of these animals is extracted and processed to produce oil with therapeutic applications[1-5] and also value for cosmetic uses[6]. Besides the usable by-products of them are the meat and eggs[7].

The nature, structure and chemistry of blood cells can determine the condition of heart as well as entire circulatory system[8]. Erythrocytes or red blood cells (RBCs) provide vital functions of oxygen transport, carbon dioxide transport and buffering of hydrogen ions[9,10]. Morphology of erythrocyte often is an important tool in diagnosing the cause of anemia, and it is helpful in establishing the diagnosis of other disorder as well[11]. Since studies on cytomorphological features of red blood cells and white blood cells (leukocytes) of the captive emus were not properly documented in literature, to know the different shapes of erythrocytes and leucocytes and to provide a baseline data of morphometry of blood cells of emus the present study is undertaken.

Materials and methods

The work was designed during the month of January, February, March and April of 2016 and four birds were taken in each month. In the present study one ml of blood sample was collected from each individual live bird without killing from jugular vein of the neck by a veterinary expert and transferred into EDTA (Ethylene Diamine Tetraacetic Acid) vials. Adequate measures were taken to minimize the discomfort and pain according to the international ethical committee.

Blood smears staining and cytomorphometrical study: The blood smears were made on clean microscopic slides. Then smears were stained with Giemsa stain prepared from Giemsa powder (Qualigens CAS NO. 51811-82-6 Product No. 39382, Thermo Fisher Scientific India Pvt. Ltd. Mumbai, Maharastra,

India) as cited by Lillie[12]. Morphometrical analyses of each cells were done using an ocular micrometer which was standardized against a stage micrometer (ERMA TOKYO, Japan made) using a standard light microscope (LABOSCOPE MICROSCOPES Research microscope M. No. BD- 08 B, S. No. 21320 Mfg. by B.D. INSTRUMENTATION, Ambala Cantt, India) under 40X objective[13]. Length and breadth of blood cells were measured in µm.

Photomicrography: Photomicrography of blood cells were done by CC130-1.3 mega pixel microscopic camera (Mfg. by Catalyst Biotech, Maharashtra, India) connected to microsope (LABOSCOPE MICROSCOPES Research microscope M. No. BD- 08 B, S. No 21320 Mfg, By B.D. Instrumentation, Ambala Cantt, India) under 40X objective.

Statistical analyses: For the study statistical calculations are applied using the software Microsoft Office Excel 2007. Results are expressed in mean.

Results and discussion

Examinations of blood are indispensable tool in bird medicine[14]. To study the qualitative and quantitative measure changes in the RBCs and WBCs fractions hematological examinations are helpful and for diagnosis of several diseases and pathologies due to changes in cell morphology[15]. Physiological condition of organism can be revealed by the cytomorphometry of blood cells[16]. The measurements of RBCs are there in literature for some species of fishes, amphibians and reptiles[17]. In the case of adult ostriches, for both in RBCs and WBCs the morphological and morphometrical features are observed[18]. Cellular and nuclear measurement of RBCs, lymphocytes and monocytes and measurement of granulocytic and thrombocytic cells are studied in different chickens[19]. From the present study it has been observed that the elliptical red blood cells have different sizes of oval to elliptical nuclei and centrally located nuclei have brown color cytoplasm (Figure-2). Round shaped immature erythrocytes are also noticed in this study (Figure-3) having irregular shaped nucleus. Lymphocyte found had a round nucleus (Figure-10). Monocyte was rounded cell (Figure-11) Heterophil (Figure-12) appeared as round cell having trilobed nucleus (Figure-12). Eosinophil was round shaped having bilobed nucleus (Figure-13) Basophil was round in emus and the nucleus was not lobed (Figure-14).

Avianery throcytes are large, nucleated and ovoid-shaped with a similarly shaped nucleus at the center and have a deep purple color with uniformly clumped chromatin having similar type of mammalian cytoplasm[20,21]. The erythrocytes were observed to occur in various forms. Avian erythrocytes need to be evaluated on the basis of shape, size, color, nucleus and presence of cellular inclusions[22]. In the observation of blood smears the erythrocytes have oval to elliptical nuclei which differ from what was reported by Blue –McLendon, and Green[23] for ratites that only found oval cells with oval nuclei, as did Pacheco, Baraldi-Artoni and Ferreira[24] for partridges (*Rhynchotus ruflescens*) and by previous author[25] for the Brazilian Duck (*Amazonetta brasiliensis*), by previous researchers[26-28] for birds, by previous worker[25] for the blackish rail (*Pardirallus nigricans*) and by Fortes et al.[29] forrhea (*R.americana*) observed only cells with shape and ellipti calnucleus what was reported by Blue –McLendon and Green[23] for ratites that they have only oval cells with oval nuclei are observed. According to Wakenell[30] avian erythrocytes are oval nucleated cells with round shaped immature RBCs. With disagreement with Blue –McLendon, and Green[23] it is observed that elliptical to oval shaped emu erythrocytes have nuclei of central position having clumped chromatin of uniform nature as were seen in ratite erythrocytes whereas like those of other avian species raptors have elliptical erythrocytes with centrally placed elongated oval nucleus as studied by Campbell et al.[31] with an exception to water fowls who have elliptical cells with centrally placed elliptical nucleus in mature erythrocytes. According to Blue –McLendon and Green[23] immature erythrocytes of ratite have a more round shape than mature erythrocytes as well as their nucleus is also more round and much less condensed than nucleus of mature erythrocyte which is congruent with our study (Figure-3).

Figure-1: Emu.

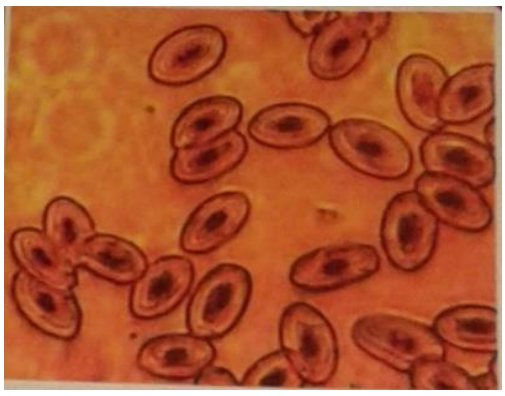

Figure-2: Elliptical RBCs with centrally placed nucleus.

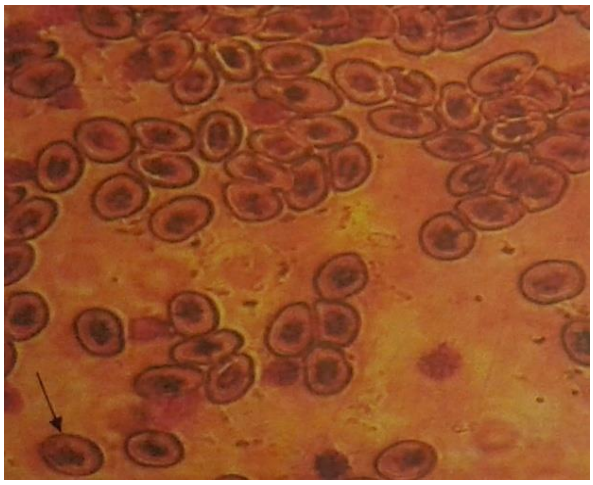

Figure-3: Circular RBCs with centrally placed nucleus indicated by small arrows.

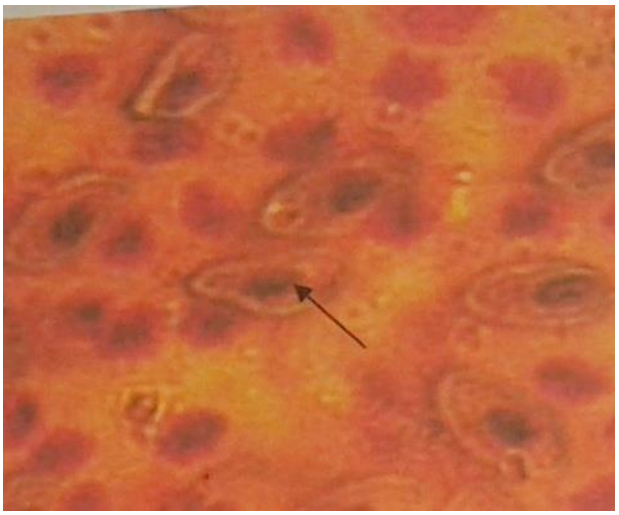

Figure-6: Tear drop shaped RBC.

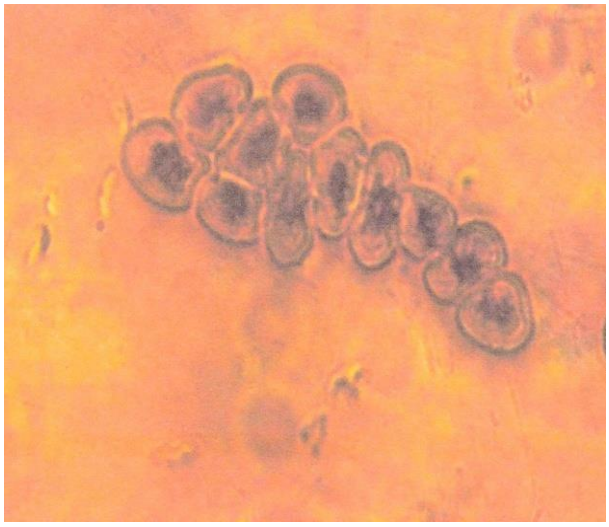

Figure-4: Rouleax formation.

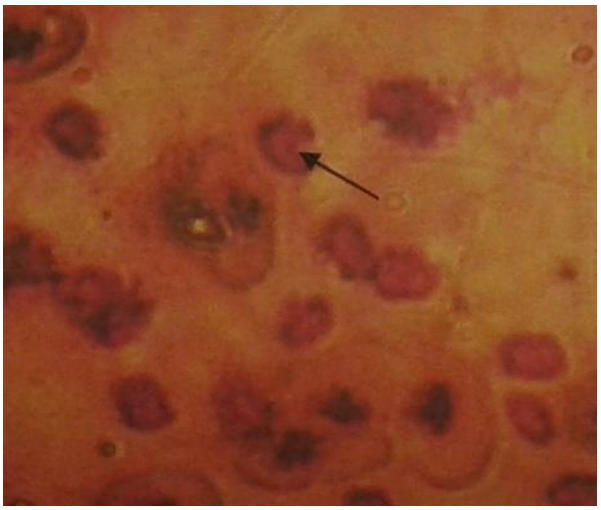

Figure-7: Fragmented RBC.

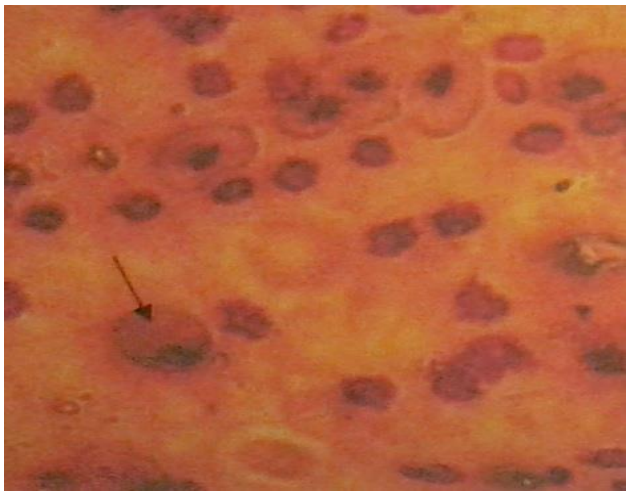

Figure-5: Circular RBC with eccentrically placed Nucleus.

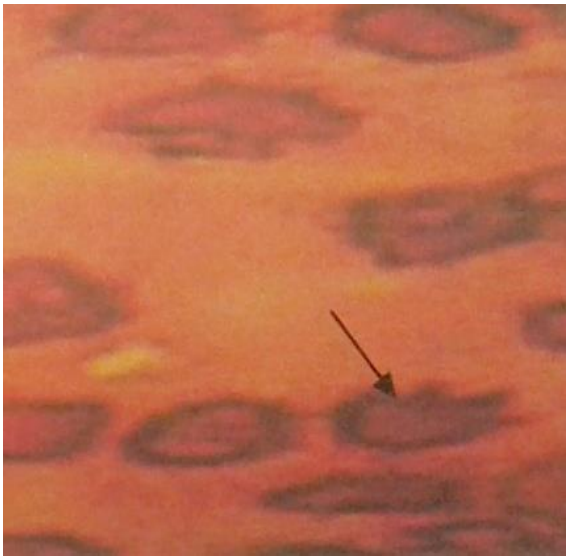

Figure-8: Acanthocyte.

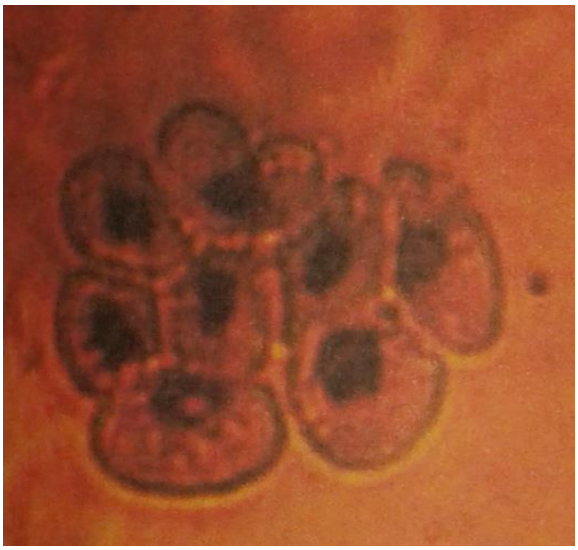

Figure-9: Clumped RBCs.

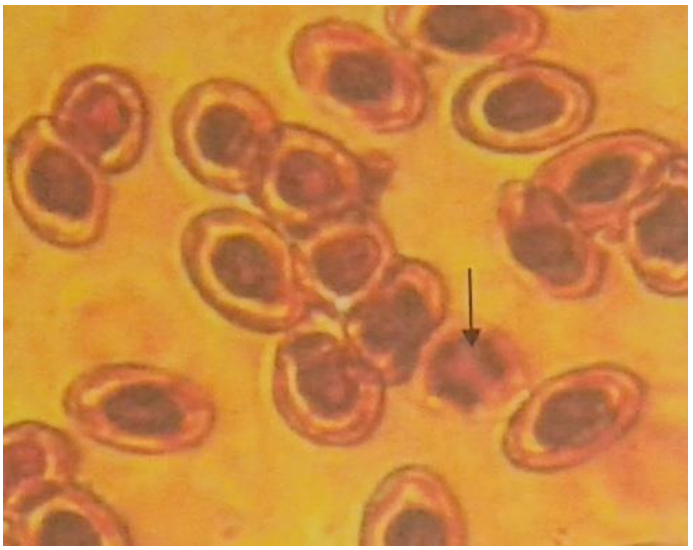

Figure-12: Heterophil.

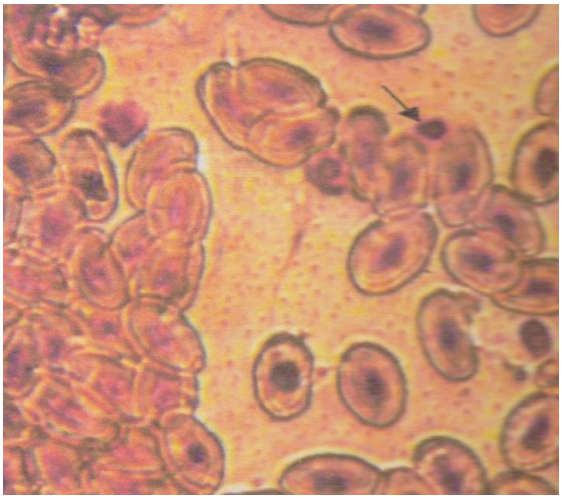

Figure-10: Lymphocyte.

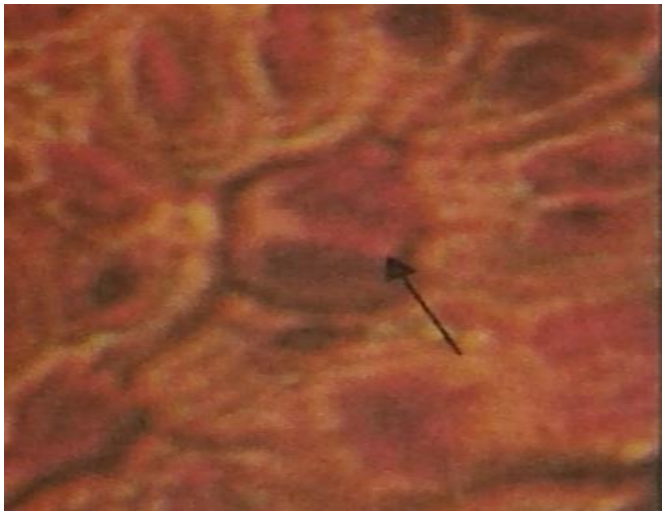

Figure-13: Eosinophil.

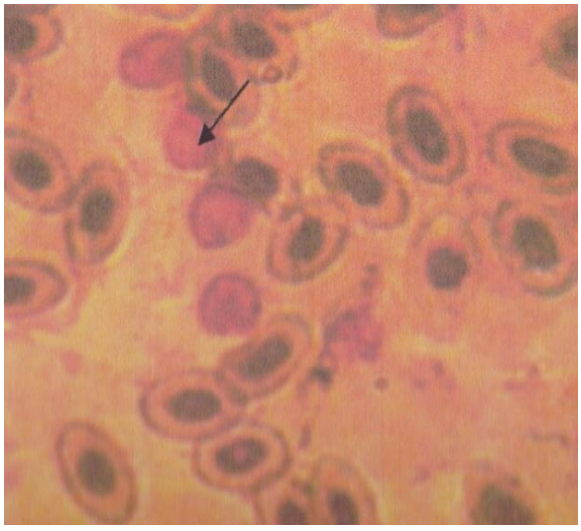

Figure-11: Monocyte.

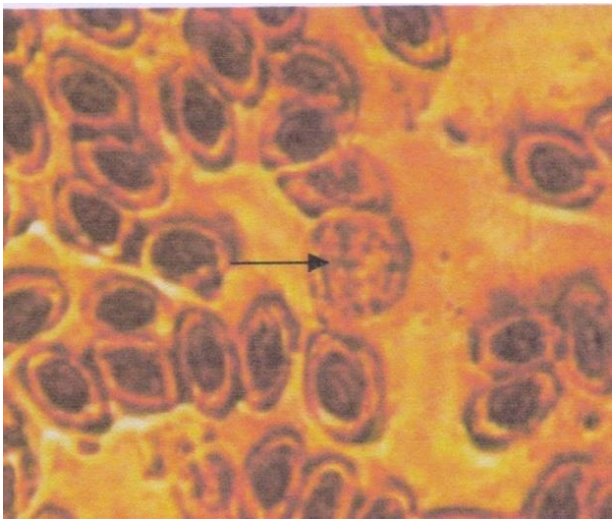

Figure-14: Basophil.

As discussed by Thrall[11] morphology of erythrocyte often is an important tool in diagnosing the cause of anemia, and it is helpful in establishing the diagnosis of other disorder as well. Poikilocytosis is a general term for variation in shape of RBC[32,10] which can occur in a variety of conditions. Therefore, poikilocytosis is non-specific[10].

The erythrocytes were observed to occur in various forms. Avian erythrocytes need to be evaluated on the basis of shape, size, color, nucleus and presence of cellular inclusions[33]. They were either elliptical in shape with centrally placed nucleus (Figure-2) or in different shapes.

The RBCs are spherical with centrally placed nucleus (Figure-3). Along with normal erythrocytes aggregated erythrocytes or rouleax formation (Figure-4), Circular RBC with eccentrically placed nucleus (Figure-5), tear drop shaped RBCs or dacryocytes (Figure-6), fragmented RBC (Figure-7). Acanthocytes (Figure-8) and clumped RBCs (Figure-9) were observed.

Presence of abnormal shaped erythrocytes is known as poikilocytosis[32,10]. Schistocytes are defined as erythrocyte fragments having pointed extremities. Appearance of erythrocyte fragments may occur when through altered vascular channels erythrocytes are forced to flow or for turbulent blood they have exposed[32]. Dacryocytes are defined as tear drop shaped erythrocytes having single pointed or elongated extremities (Figures-5 and 6)[32]. In adult ostriches, for both in RBCs and WBCs the morphological and morphometrical features are observed[33].

Figure-4 is not in accordance with Lucas and Jamroz[19] who stated that no clumping of thrombocytes and no rouleax formation was reported in avian blood, hence no segregation observed.

In these present study five types of leucocytes namely lymphocyte, monocyte, heterophil, eosinophil and basophil were distinguished and characterized. The measurement of blood cells with nuclei are done (Table-1).

The highest mean lengths of RBCs (15.28±0.86) were observed in the month of April and lowest (10.92±0.52) in February. The highest mean breadths (10.29±0.47) of RBCs were observed in March and April and lowest (7.8±0.52) in February. The highest mean lengths of nucleus of RBCs (8.11±0.50) were observed in March and lowest (6.24±2.96) was observed in January. The highest mean breadths of nuclei of RBCs (4.99±0.50) were observed in March and lowest (3.43±0.31) was observed in January.

The highest mean lengths of lymphocyte (10.60±0.50) were observed in March and lowest (9.36±1.23) was observed in January and February. The highest mean breadth (8.42±0.47) was observed in March and lowest (8.42±0.47) in February. The highest mean length of monocytes (11.85±0.62) was observed in April and lowest (8.11±0.50) was observed in February. The highest mean breadth of monocyte (9.98±0.41) was observed in February and lowest (8.73±0.62) in January. The highest mean length of heterophil (11.54±2.56) was observed in March and lowest (9.04±0.31) in February. The highest mean breadth of heterophil (9.67±1.04) was observed in March and lowest (8.11±0.50) in February.

The highest mean length of eosinophil (14.29±0.95) was observed in January and lowest (9.67±0.56) in February. The highest mean breadth of eosinophil (9.67±0.98) was observed in March and lowest (8.73±0.62) in both January and February. The highest mean length of basophil (12.48±0.80) was observed in April and lowest (8.73±0.41) in January. The highest mean breadth of basophil (10.60±0.68) was observed in April and lowest (6.86±0.41) in January.

According earlier authors[20,21] size range of erythrocyte of bird varies, having length 11 to 16 µm and breadth 6 to 10µm which is in accordance with our study except in the month of February 2016.

As stated by one author, among the avian species the largest erythrocytes are observed in Rheiformes[34]. Our study is in agreement with previous researchers[19,35-37] who stated that heterophils measure about 5.1 to 11.4µm. According to Campbell and Dein[36] eosinophils measure 4-11µm in diameter which corroborates with present study except January 2016.

This study corroborates with Coles et. al.[38] who stated that the lymphocytes measuring 6 to 12µm. Basophils measures about 4.9 to 19.9µm in diameter which is observed in our study[38]. In captive breeding from Brazil study of blood cells of adult rheas, *Rhea americana* was done by earlier authors[40] who observed that erythrocyte length and width was 14.03±1.04 and 8.47±0.89 respectively, lymphocytes length and width was 8.71±1.92 and 7.44±1.65 respectively, monocytes length and width was 15.62±2.64 and 13.50±2.16 respectively, heterophils length and width was 12.93±1.57 and 11.56±1.26 respectively , eosinophils length and width was 13.04±1.69 and 11.15±1.35 respectively and basophils length and width was 11.49±1.24 and 10.32±1.06 respectively.

Conclusion

Poikilocytosis is observed in emus. Seasons have profound effect on the mean morphometry values of blood cells of emus and physiological condition of them can be known by morphometrical analyses of blood cells.

The different values observed are may be due to their age, sex, body weight, physiological changes and environmental influence. The present data has also established a base line to which further studies may be compared.

Table-1: Mean blood cell morphometry (in µm) of emus (*Dromaius novaehollandiae*).

Type of cell	Cell/Nucleus	Parameters	January (2016)	February (2016)	March (2016)	April (2016)
Erythrocyte	Cell	Length	14.04	10.92	14.66	15.28
		SEM	0.52	0.52	0.66	0.86
		Breadth	9.36	7.8	10.29	10.29
		SEM	0.46	0.52	0.47	0.47
	Nucleus	Length	6.24	6.84	8.11	7.48
		SEM	2.96	0.41	0.50	0.50
		Breadth	3.43	3.74	4.99	3.74
		SEM	0.31	0.41	0.50	0.41
Lymphocyte	Cell	Length	9.36	9.36	12.79	10.60
		SEM	1.23	1.23	0.56	0.50
		Breadth	8.73	8.42	12.16	9.67
		SEM	0.90	0.47	0.56	0.77
Monocyte	Cell	Length	9.98	8.11	8.73	11.85
		SEM	0.77	0.50	1.01	0.62
		Breadth	8.73	9.98	8.73	9.04
		SEM	0.62	0.41	3.83	2.30
Heterophil	Cell	Length	10.29	9.04	11.54	9.36
		SEM	0.66	0.31	2.56	2.08
		Breadth	8.73	8.11	9.67	8.42
		SEM	0.90	0.50	1.04	0.81
Eosinophil	Cell	Length	14.29	9.67	10.92	10.29
		SEM	0.95	0.56	0.83	0.66
		Breadth	8.73	8.73	9.67	9.04
		SEM	0.6	0.62	0.98	0.98
Basophil	Cell	Length	8.73	10.92	10.92	12.48
		SEM	0.41	0.52	0.69	0.80
		Breadth	6.86	9.36	10.60	10.60
		SEM	0.41	0.65	0.50	0.68

SEM- Standard error mean

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