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Morphometric analysis on erythrocytes of various livestock being reared in the Cholistan desert

[*Análise morfométrica de eritrócitos de vários pecuários criados no deserto de colistão*]

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ABSTRACT

The present study aimed to assess the influence of physiological exposure to erythrocyte indices; the morphometric parameters of the size of erythrocytes, as well as synergistic effects on the erythrocyte spectrum changes in various species being reared in different strip-wasteland conditions. We collected samples from 400 animals from the jugular veins and made 2000 blood smears, sample blood was taken by an expert veterinary doctor and transferred into an EDTA vacutainer. Blood smears were stained through the Pappenheim procedure and slides were photomicrographed under 100x objective. The parameters of morphometry of erythrocytes were largest in cattle after buffalo, sheep, while goats have the lowest one. The influence of age was significant $p<0.05$. The influence of sex was not significant in Cattle $p<0.01$, values of a parameter of erythrocytes were higher in young than in adults in sheep, goats, cattle, and buffalo. The size of erythrocytes in males was higher than females in sheep, goat, and buffalo but in cattle values of female erythrocytes were higher than in males. Further, gender, altitude, and age have a profound influence on the morphometric attributes of erythrocytes. It will be helpful in interpreting etiopathogenetic conditions in human beings and other animals.

Keywords: Erythrocyte morphometry, livestock, gender, age, altitude

RESUMO

O presente estudo teve como objetivo avaliar a influência da exposição fisiológica nos índices eritrocitários; os parâmetros morfométricos do tamanho dos eritrócitos, bem como os efeitos sinérgicos sobre as alterações do espectro eritrocitário em várias espécies criadas em diferentes condições de terreno baldio. Coletamos amostras de 400 animais das veias jugulares e fizemos 2.000 esfregaços de sangue, a amostra de sangue foi colhida por um médico veterinário especialista e transferida para um vacutainer de EDTA. Esfregaços de sangue foram corados pelo procedimento de Pappenheim e as lâminas foram fotomicrografadas sob objetiva de 100x. Os parâmetros de morfometria dos eritrócitos foram maiores em bovinos após bubalinos, ovinos, enquanto os caprinos apresentaram os menores. A influência da idade foi significativa $p<0,05$. A influência do sexo não foi significativa em Bovinos $p<0,01$, os valores de um parâmetro de eritrócitos foram maiores em jovens do que em adultos em ovinos, caprinos, bovinos e bubalinos. O tamanho dos eritrócitos nos machos foi maior que nas fêmeas em ovinos, caprinos e búfalos, mas em bovinos os valores de eritrócitos femininos foram maiores do que nos machos. Além disso, sexo, altitude e idade têm uma profunda influência nos atributos morfométricos dos eritrócitos. Será útil na interpretação de condições etiopatogenéticas em seres humanos e outros animais.

Palavras-chave:morfometria de eritrócito, rebanho, sexo, idade, altitude

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INTRODUCTION

Erythrocytes deliver oxygen to the body's organs, extract outside carbon dioxide, and buffer H^+ ions (Dash, 2020). Erythrocytes have no organelles, no nuclei, and have no ability to make proteins (Thamer *et al*., 2016). Erythrocytes are the largest biological component in circulating blood. The number of erythrocytes in circulation is roughly 05 million per cubic millimeter of blood (Samson Adewoyin *et al*., 2019). An increase in erythrocyte concentration was observed for species exposed to direct sunlight. Erythrocytes are concave on both sides enucleate that transport oxygen/carbon dioxide between lungs and body tissues, their high hemoglobin concentration adds to their O_2 capacity (Menon and Ghaffari, 2021).

The breeds of goat *(Capra aegagrus hircus)*, sheep *(Ovis aries)*, cattle *(Bos taurus indicus)*, and buffalo *(Bubalus bubalis)* are one of the most domesticated livestock that are extensively found and commonly raised in a huge desert situated in the Bahawalpur region of Punjab, Pakistan, situated on the southwest side of the province and located at a longitude of 57 to 60 east and latitude of 27 to 40 north (Type *et al*., 2021). It is at a level of 112 meters above sea level (Ali *et al*., 2009). It has a vast significance in culture, economy, and agriculture from the era of the Neolithic agricultural insurgency (Naderi *et al*., 2008).

Erythrocytes of Angora breed goat are discoid with the 2.5 to 3.9 µm of width, which normally has a greater fraction of fusiform shaped erythrocytes which is assorted in discoid erythrocytes (Kramer, 2000). Erythrocytes of ovine have a lifespan of 70-150 days having a width of 3.2-5µm (Thamer *et al*., 2016). A Caprine erythrocyte has a lifespan of 125 days and a width of 2.5-3.9µm, which is commonly discoid (Mariella *et al*., 2014). A breed of cattle zebu or humped breed is of Indian origin like other zebu cattle found in Pakistan. The literature was reviewed with respect to the morphometry of erythrocytes and the influences with respect to age, sex, and species. Various authors have studied the influence of age (Adili *et al*., 2013; Mohanty, 2015), sex (Adili *et al*., 2013), and breed (Adili *et al*., 2014; Mohanty, 2015) on the morphometry of erythrocytes. Gender, age, altitude, physiological state, seasonal and diurnal change, feeding level, breed, and temperature of animals all contribute to variations in blood parameters (Mbassa and Poulsen, 1993).

This work is based on the hypothesis that the selected areas cause specific changes in the surface roughness, stiffness, and morphology (area (a), length (l), width (w), perimeter (p), height (h), ferret (f), roundness (r), and solidity (s)) of erythrocytes. We deployed optical microscopy (OM) to establish specific morphometric features and alterations in the male, female, young, and adult modules of erythrocytes from different altitudes and their dependence on the cell size. We found considerable differences in the gender, altitude, and age distribution of the size of erythrocytes derived from discussed species in the three studied regions. It is envisaged that the present study will assist to give information about the anemic syndrome and species type with the drop of blood measuring the parameters of the size of the erythrocytes.

MATERIALS AND METHODS

The experimental animals comprised Cholistani breeds of goat (beetal), sheep (kajli), cattle (zebu), and buffalo (Nili-Ravi) belonging to private or government livestock farms or nomadic pastoralists having the age between 1-6 months of young, and 14-50 months for adult; for goat and sheep, 1-10 month for young, and 30-60 for adult cattle and buffalo, respectively, with 4 groups of each species that are male, female, young, and adult groups. A total of 400 blood samples with 1 of each species with 5 blood smears from each sample, a total of 2000 blood smears, livestock were randomly taken from three different regions A Khairprur Tamewali (KPT), B Derawar (DWR), and C Yazman (YZN) of Cholistan.

The climate of this area is a hot, dry subtropical, continental type with low and irregular rainfall (100-150 mm), high temperature (50^0C), annual evapotranspiration rate (300cm), low relative humidity, high rate of disappearance, and intense summer winds. The region of KPT, DWR, and YZN is one of the driest and hottest areas in the Cholistan, situated at 130m, 106m, and 115m, respectively, above sea level with a mean annual temperature of 28.33^0C.

Blood sampling was done aseptically through a disposable syringe from the jugular vein of all these animals. About 5ml of blood samples were taken from each animal. Samples of blood were venipuncture from the jugular vein (Ledieu, 2003) by a veterinary doctor. Before the venipuncture, hair on the site of puncture was trimmed off and the skin was disinfected according to the perspective of the standard veterinary procedure. The storage capacity for blood samples was at +4 ^{0}C and applied procedures in the laboratory on the very same day of the collection of the blood (Žaja *et al*., 2019). The blood sample was shifted into a screw-capped tube which has 0.5ml of 1% EDTA (ethylene diamine tetra-acetic acid) solution as an anticoagulant. A mixture of classical panoptic staining of the Pappenheim procedure was carried out instead of the dye of May-Grunwald-Giemsa (Infectious, 2001) (Romanowsky type) stain field A which is methylene blue and Azure1 dissolved in phosphate buffer solution. While another one is a stain field B, which is Eosin Y in buffer solution was used, which is the best and very suitable procedure of staining to spot the erythrocytes of mammals in the laboratory (Ledieu, 2003). A high-performance optical microscope, named "OPTIKA 4083.B3" was used. Microscopic pictures were selected and then it was opened in ImageJ and a set scale was done and results were analyzed. The statistical analysis was examined by using the student's t-test for the subject of gender and age, while ANOVA was used for altitude. To better describe the results, we use Python for the graph, module name ". matplotlib" (Ucuzal *et al*., 2019).

RESULTS

Table 1 showed that a slight difference is present in the three different regional sites in the area and perimeter of erythrocytes, a significant difference is present in shape parameters (roundness and solidity) between the KPT and DWR but there was no difference between the values of solidity in DWR and YAZ. It seems that the altitude can affect the parameters of the size of erythrocytes in sheep, goats, buffalo, and cattle. Climatic conditions like temperature, pressure, moisture, etc., could cause a change in blood morphology.

Table 1. Morphometric parameters (area, length, width, perimeter, height, ferret, roundness, and solidity) of size of erythrocytes in three regions (KPT, DWR and YZN) of livestock. (All results expressed in microns) (N=400) (Sample smear=2000)

Parameters		**(a)**	**(l)**	**(w)**	**(p)**	**(h)**	**(f)**	**(r)**	**(s)**
Goat	KPT	6.92	1.71	2.88	8.84	2.74	2.99	0.89	1.003
	DWR	7.97	1.57	3.27	9.96	3.05	3.38	0.88	1.001
	YZN	8.54	1.64	3.31	10.26	3.21	3.40	0.92	1.001
	Avg	**7.81**	**1.64**	**3.16**	**9.69**	**3.00**	**3.26**	**0.89**	**1.002**
Sheep	KPT	9.06	3.09	3.40	10.56	3.32	3.54	0.90	1.002
	DWR	9.60	3.17	3.48	10.84	3.42	3.64	0.90	1.002
	YZN	10.34	3.25	3.64	11.31	3.55	3.77	0.91	1.002
	Avg	**9.67**	**3.17**	**3.51**	**10.90**	**3.43**	**3.65**	**0.90**	**1.002**
Cattle	KPT	16.98	4.91	4.64	14.58	4.63	4.81	0.93	1.003
	DWR	18.41	4.71	4.87	15.16	4.78	5.01	0.93	1.002
	YZN	17.72	4.70	4.73	14.86	4.73	4.90	0.93	1.003
	Avg	**17.71**	**4.77**	**4.75**	**14.87**	**4.71**	**4.91**	**0.93**	**1.003**
Buffalo	KPT	16.60	4.30	4.60	14.40	4.60	4.80	0.92	1.003
	DWR	16.10	4.30	4.50	14.20	4.50	4.70	0.91	1.004
	YZN	16.50	4.20	4.50	14.40	4.60	4.80	0.91	1.004
	Avg	**16.40**	**4.30**	**4.60**	**14.30**	**4.60**	**4.80**	**0.91**	**1.003**

Table. 2 shows the influence of caprine sex on erythrocytic size with an increase in females as compared to males. It is pertinent to mention and noted that all the differences are significant ($p<0.01$) in the size of erythrocytes, while they are highly significant in shape, where $p<0.001$ and $p<0.0001$. Certainly, morphometry of the erythrocytes of caprine gender does not have any influence in our study. The results exist within the normal physiological range cited by different

authors. These results showed that there is a significant difference ($p<0.001$) present in the values of area, perimeter, and round parameters between the male and female in sheep erythrocytes, while in cattle, values of parameters of erythrocytes are lower in females than in males, however, the values of parameters of the size of erythrocytes in male buffalos are higher than females.

Table 2. Morphometric parameters (area, length, width, perimeter, height, ferret, roundness, and solidity) of size of erythrocytes with respect to gender (male and female) of livestock. (All results expressed in microns) (N=400) (Sample smear=2000)

Goat				
Parameters	Male (M ± S.D) (N=50)	SEM	Female (M ± S.D) (N=50)	SEM
Area	7.583 ± 3.496	0.221	7.854 ± 3.350	0.212
Length	1.665 ± 0.551	0.035*	1.625 ± 0.607	0.038*
Width	3.095 ± 0.833	0.053*	3.159 ± 0.788	0.050*
Perimeter	9.503 ± 2.405	0.152	9.698 ± 2.305	0.146
Height	2.943 ± 0.773	0.049*	3.005 ± 0.749	0.047*
Ferret	3.206 ± 0.824	0.052*	3.256 ± 0.778	0.049*
Round	0.889 ± 0.093	0.00586**	0.896 ±0.090	0.00568**
Solidity	1.001 ± 0.007	0.00045***	1.002 ± 0.007	0.00041***
Sheep				
Area	10.22 ± 3.21	0.203	9.044 ± 3.210	0.203
Length	3.32 ± 0.63	0.040*	3.003 ± 0.691	0.044*
Width	3.602 ± 0.640	0.040*	3.396 ± 0.620	0.039*
Perimeter	11.22 ± 1.769	0.112	10.538 ± 1.812	0.115
Height	3.54 ± 0.574	0.036*	3.303 ± 0.629	0.040*
Ferret	3.750 ± 0.588	0.037*	3.537 ± 0.587	0.037*
Round	0.907 ± 0.082	0.00521**	0.895 ± 0.082	0.00519**
Solidity	1.00 ± 0.004	0.00023***	1.002 ± 0.004	0.00028***
Cattle				
Area	17.3 ± 3.8	0.2677188	18.2 ± 2.9	0.02030829*
Length	4.8 ± 0.5	0.0327902*	4.7 ± 0.5	0.0351429*
Width	4.7 ± 0.6	0.0413724*	4.8 ± 0.4	0.0302496*
Perimeter	14.7 ± 1.6	0.11118029	15.1 ± 1.2	0.00857117**
Height	4.6 ± 0.5	0.034929*	4.8 ± 0.5	0.0333956*
Ferret	4.8 ± 0.5	0.038851*	5.0 ± 0.4	0.0300304*
Round	0.9 ± 0.0	0.0035161**	0.9 ± 0.1	0.0039377**
Solidity	1.0 ± 0.0	0.00021841***	1.0 ± 0.0	0.0002123***
Buffalo				
Area	16.50 ± 2.68	0.19	16.35 ± 3.08	0.218
Length	4.40± 0.52	0.037*	4.29 ± 0.54	0.038*
Width	4.60 ± 0.50	0.036*	4.53 ± 0.50	0.035*
Perimeter	14.40 ± 1.17	0.083*	14.29 ± 1.36	0.097*
Height	4.60 ± 0.45	0.032*	4.56 ± 0.51	0.037*
Ferret	4.81 ± 0.47	0.034*	4.74 ± 0.49	0.035*
Round	0.928 ± 0.0716	0.0051**	0.918 ± 0.06	0.004725**
Solidity	1.0033 ± 0.0034	0.000244***	1.0031 ± 0.00	0.000249***

M=Mean, S. D=Standard Deviation, SEM=Standard Error Mean, *=p<0.01, **=p<0.001, ***=p<0.0001

Variations in the influence of the age of species were observed very keenly (Table. 3). It was more obvious that the values of area and perimeter in young are higher than that in adults, similarly, the other parameters of erythrocytes of goats are also bigger in young than in adults. It indicates that the effect of age on caprine (male and female) size of erythrocytes is larger in young goats than in adults ($p<0.001$). Two characteristic components were attained with the help of Principal Component Analysis (PCA) and Cluster Analysis (CA) of erythrocytes

morphometry with an Eigen number greater than one (1). Variations on the influence of the age of sheep were studied by the Mann-Whitney test, which showed a highly significant difference ($p<0.001$) was present between young and adult sheep. The influence of the age of cattle is higher in adults as compared to the young; a significant difference of $p<0.01$ lies between the different parameters of young and adults. These results determine that the parameters of erythrocytes have remained higher in DWR as compared to the other two regions. Regarding variations on the effect of the age of buffaloes of erythrocytes, results showed a lack of any significant differences ($P>0.01$) between adult and young buffaloes.

Table 3. Morphometric parameters (area, length, width, perimeter, height, ferret, roundness, and solidity) of size of erythrocytes with respect to age (young and adult) of livestock. (All results expressed in microns) (N=400) (Sample smear=2000)

Goat				
Parameters	Young (M ± S.D) (N=50)	SEM	Adult (M ± S.D) (N=50)	SEM
(a)	8.358 ± 3.846	0.243	7.079 ± 2.804	0.177
(l)	1.604 ± 0.619	0.039*	1.686 ± 0.536	0.034*
(w)	3.241 ± 0.878	0.056*	3.014 ± 0.720	0.046*
(p)	9.937 ± 2.624	0.166	9.264 ± 2.001	0.127
(h)	3.078 ± 0.850	0.054*	2.870 ± 0.645	0.041*
(f)	3.328 ± 0.886	0.056*	3.134 ± 0.693	0.044*
(r)	0.903 ± 0.085	0.00539**	0.882 ± 0.096	0.00607**
(s)	1.003 ± 0.007	0.00045***	1.001 ± 0.006	0.00040***
Sheep				
(a)	10.259 ± 3.208	0.203	9.013 ± 3.204	0.203
(l)	3.248 ± 0.687	0.043*	3.083 ± 0.671	0.042*
(w)	3.592 ± 0.649	0.041*	3.406 ± 0.614	0.039*
(p)	11.244 ± 1.773	0.112	10.519 ± 1.801	0.114
(h)	3.563 ± 0.570	0.036*	3.282 ± 0.624	0.039*
(f)	3.760 ± 0.597	0.038*	3.528 ± 0.575	0.036*
(r)	0.906 ± 0.082	0.00517**	0.896 ± 0.083	0.00524**
(s)	1.002 ± 0.004	0.00023***	1.002 ± 0.004	0.00028***
Cattle				
(a)	17.6 ± 3.4	0.2319339	18.0 ± 3.3	0.2473514
(l)	4.8 ± 0.5	0.034135*	4.7 ± 0.4	0.0331501*
(w)	4.7 ± 0.4	0.033437*	4.8 ± 0.5	0.0393556*
(p)	14.8 ± 1.4	0.096236*	15.0 ± 1.4	0.1046301
(h)	4.7 ± 0.4	0.033503*	4.7 ± 0.4	0.0367096*
(f)	4.9 ± 0.4	0.032709*	5.0 ± 0.5	0.0376437*
(r)	0.9 ± 0.0479	0.003231**	0.9 ± 0.04	0.0042907**
(s)	1.0 ± 0.0031	0.000209***	1.0 ± 0.0029	0.0002213***
Buffalo				
(a)	16.3 ± 2.97	0.2101	16.6 ± 2.8	0.1983
(l)	4.2 ± 0.51	0.0367*	4.3 ± 0.038	0.0835*
(w)	4.5 ± 6.80	0.0369*	4.6 ± 0.080	0.0340*
(p)	14.3 ± 1.32	0.0940*	14.4 ± 0.021	0.0864*
(h)	4.6 ± 0.51	0.0363*	4.6 ± 0.058	0.0324*
(f)	4.8 ± 0.50	0.0360*	4.8 ± 0.062	0.0327*
(r)	0.9 ± 0.0748	.0052**	0.9 ± .632	0.0044**
(s)	1.0 ± 0.00343	.000243***	1.0 ± .0349	0.000246***

M=Mean, S. D=Standard Deviation, SEM=Standard Error Mean, *=p<0.01, **=p<0.001, ***=p<0.0001

Hypoxia occurs at high altitudes. The increase in the size of a erythrocyte causes hypoxia, which leads to the reduction in an erythropoiesis time period, the erythrocyte size is higher in the DWR region followed by YZN and KPT regions. As DWR is so hot, the dry region, water, and

vegetation depend on rainfall which is very low. In the case of buffalo, the values of erythrocyte parameters remained higher in KPT as compared to the other two regions (Table. 4).

Table 4. Morphometric parameters (area, length, width, perimeter, height, ferret, roundness, and solidity) of size of erythrocytes of species (100 blood smears from every specie with 500 measured erythrocytes, 5 from each blood smear) distributed randomly in three distinct regions (KPT, DWR, YZN) with respect to age (young, adult) and gender (male, female) of livestock. (All results expressed in microns). (N=400) (Sample smear=2000)

Goat												
Regions	Gender	Age	N	Samples	(a)	(l)	(w)	(p)	(h)	(f)	(r)	(s)
KPT	Male	Young	10	50	7.98	1.72	3.05	9.32	2.87	3.14	0.89	1.005
		Adult	10	50	5.86	1.69	2.71	8.36	2.60	2.83	0.88	1.001
	Female	Young	10	50	7.98	1.69	3.05	9.32	2.87	3.14	0.89	1.005
		Adult	10	50	5.86	1.72	2.71	8.36	2.60	2.83	0.88	1.001
DWR	Male	Young	10	50	7.38	1.48	3.14	9.60	2.96	3.26	0.88	1.001
		Adult	5	25	9.20	2.07	3.56	10.72	3.25	3.66	0.87	1.002
	Female	Young	5	25	8.40	1.38	3.27	10.24	3.24	3.41	0.91	1.002
		Adult	10	50	7.72	1.51	3.27	9.80	2.95	3.36	0.86	1.001
YZN	Male	Young	5	25	10.17	1.43	3.74	11.31	3.46	3.78	0.91	1.002
		Adult	10	50	7.01	1.69	2.93	9.22	2.94	3.08	0.91	0.999
	Female	Young	10	50	9.16	1.72	3.45	10.68	3.34	3.51	0.94	1.002
		Adult	5	25	8.70	1.57	3.36	10.43	3.27	3.46	0.92	1.001
Total Sheep			100	500	7.95	1.64	3.19	9.78	3.03	3.29	0.89	1.002
KPT	Male	Young	10	50	8.73	2.98	3.32	10.39	3.30	3.51	0.89	1.001
		Adult	5	25	7.92	3.03	3.27	9.98	3.07	3.35	0.90	1.000
	Female	Young	10	50	11.85	3.55	3.84	12.11	3.86	3.99	0.93	1.003
		Adult	10	50	7.17	2.77	3.10	9.46	2.91	3.22	0.87	1.002
DWR	Male	Young	10	50	9.15	3.19	3.36	10.56	3.37	3.56	0.89	1.001
		Adult	10	50	12.29	3.74	3.95	12.38	3.92	4.08	0.93	1.003
	Female	Young	5	25	11.17	3.35	3.84	11.79	3.66	3.95	0.90	1.004
		Adult	10	50	6.58	2.50	2.94	9.11	2.85	3.11	0.87	1.001
YZN	Male	Young	5	25	10.72	3.19	3.77	11.58	3.59	3.90	0.89	1.001
		Adult	10	50	11.64	3.63	3.86	12.02	3.78	3.98	0.92	1.002
	Female	Young	10	50	10.61	3.26	3.64	11.47	3.66	3.81	0.92	1.003
		Adult	5	25	6.84	2.52	3.07	9.30	2.83	3.16	0.88	1.002
Total Cattle			100	500	9.56	3.14	3.50	10.85	3.40	3.63	0.90	1.002
KPT	Male	Young	6	30	16.21	4.85	4.53	14.26	4.54	4.65	0.95	1.003
		Adult	4	20	16.38	4.91	4.62	14.32	4.49	4.73	0.92	1.001
	Female	Young	6	30	16.87	4.86	4.58	14.53	4.67	4.79	0.93	1.003
		Adult	4	20	18.91	5.08	4.93	15.42	4.87	5.15	0.91	1.003
DWR	Male	Young	6	30	18.51	4.79	4.83	15.15	4.80	4.97	0.94	1.002
		Adult	6	30	18.35	4.71	4.96	15.11	4.65	5.00	0.93	1.003
	Female	Young	6	30	17.78	4.70	4.79	14.91	4.70	4.93	0.93	1.001
		Adult	8	40	18.86	4.66	4.88	15.41	4.91	5.12	0.92	1.003
YZN	Male	Young	10	50	17.85	5.01	4.76	14.91	4.73	4.92	0.93	1.003
		Adult	8	40	16.12	4.48	4.61	14.17	4.41	4.72	0.92	1.002
	Female	Young	10	50	17.75	4.59	4.70	14.88	4.76	4.90	0.93	1.002
		Adult	6	30	19.61	4.65	4.90	15.68	5.08	5.12	0.95	1.002
Total Buffalo			80	400	17.77	4.77	4.76	14.90	4.72	4.92	0.93	1.003
KPT	Male	Young	6	30	17.24	4.25	4.68	14.76	4.69	4.99	0.89	1.003
		Adult	6	30	17.12	4.48	4.69	14.65	4.63	4.90	0.91	1.002
	Female	Young	6	30	16.60	4.32	4.53	14.38	4.62	4.70	0.95	1.005
		Adult	6	30	15.54	4.22	4.52	13.98	4.37	4.62	0.93	1.000
DWR	Male	Young	4	20	17.59	4.43	4.74	14.87	4.71	5.02	0.89	1.003
		Adult	6	30	16.10	4.34	4.52	14.20	4.52	4.71	0.92	1.003
	Female	Young	4	20	15.30	3.94	4.42	13.81	4.36	4.62	0.90	1.004
		Adult	6	30	15.79	4.30	4.48	14.06	4.46	4.68	0.91	1.005
YZN	Male	Young	10	50	15.68	4.13	4.35	14.03	4.57	4.69	0.91	1.004
		Adult	8	40	16.47	4.06	4.59	14.38	4.56	4.76	0.93	1.004
	Female	Young	10	50	16.16	4.26	4.48	14.22	4.56	4.74	0.91	1.004
		Adult	8	40	17.98	4.53	4.72	15.00	4.82	5.01	0.91	1.004
Total			80	400	16.47	4.27	4.56	14.36	4.57	4.78	0.91	1.003

The statistical values of the various parameters of morphometry of size and shape of erythrocytes in livestock are shown in Table 5. The parameters of shape are roundness and solidity. All the values of all the parameters of size and shape were significant. These values are according to (Adili *et al*., 2016) and remained in international standards.

Table 5. Statistical values of morphometric parameters of livestock

Parameters	Species	Avg	Min	Max	S. D	SEM	Range	Median	Sum	Mean
(a)	Goat	7.719	1.180	18.100	3.426	0.153	16.920	7.784	3859.369	7.719
	Sheep	9.636	4.620	20.980	3.266	0.146	16.360	8.813	4817.968	9.636
	Cattle	17.763	8.754	30.620	3.394	0.170	21.866	17.640	7105.084	17.763
	Buffalo	16.449	9.418	26.980	2.893	0.145	17.562	16.310	6579.622	16.449
(l)	Goat	1.645	0.567	2.511	0.580	0.026	1.944	1.556	822.540	1.645
	Sheep	3.165	1.939	5.343	0.684	0.031	3.404	3.033	1582.748	3.165
	Cattle	4.755	3.220	6.661	0.482	0.024	3.441	4.868	1902.064	4.755
	Buffalo	4.270	2.677	5.906	0.535	0.027	3.229	4.248	1708.005	4.270
(w)	Goat	3.127	1.090	4.920	0.811	0.036	3.830	3.272	1563.634	3.127
	Sheep	3.499	2.065	5.710	0.639	0.029	3.645	3.523	1749.540	3.499
	Cattle	4.753	3.402	6.682	0.514	0.026	3.280	4.738	1901.250	4.753
	Buffalo	4.548	3.280	6.804	0.504	0.025	3.524	4.495	1819.361	4.548
(p)	Goat	9.600	3.830	15.100	2.358	0.105	11.270	9.950	4800.065	9.600
	Sheep	10.882	7.653	16.220	1.823	0.082	8.567	10.630	5440.793	10.882
	Cattle	14.891	10.500	19.680	1.421	0.071	9.180	14.900	5956.480	14.891
	Buffalo	14.355	10.880	18.710	1.278	0.064	7.830	14.380	5742.130	14.355
(h)	Goat	2.974	1.220	4.980	0.762	0.034	3.760	3.040	1487.009	2.974
	Sheep	3.422	2.187	5.224	0.614	0.027	3.037	3.280	1711.140	3.422
	Cattle	4.721	3.280	6.561	0.495	0.025	3.281	4.738	1888.280	4.721
	Buffalo	4.582	3.159	6.439	0.487	0.024	3.280	4.617	1832.616	4.582
(f)	Goat	3.231	1.340	4.980	0.802	0.036	3.640	3.280	1615.357	3.231
	Sheep	3.644	2.551	5.710	0.597	0.027	3.159	3.523	1821.793	3.644
	Cattle	4.914	3.402	6.682	0.497	0.025	3.280	4.860	1965.633	4.914
	Buffalo	4.780	3.523	6.804	0.487	0.024	3.281	4.738	1912.012	4.780
(r)	Goat	0.893	0.539	1.000	0.091	0.004	0.461	0.917	446.289	0.893
	Sheep	0.901	0.576	1.000	0.082	0.004	0.424	0.917	450.413	0.901
	Cattle	0.930	0.739	1.000	0.053	0.003	0.261	0.934	371.849	0.930
	Buffalo	0.914	0.670	1.000	0.069	0.003	0.330	0.930	365.454	0.914
(s)	Goat	1.002	0.976	1.050	0.007	0.000	0.074	1.000	500.840	1.002
	Sheep	1.002	0.989	1.020	0.004	0.000	0.032	1.001	500.910	1.002
	Cattle	1.003	0.996	1.012	0.003	0.000	0.016	1.002	401.026	1.003
	Buffalo	1.003	0.995	1.013	0.003	0.000	0.018	1.003	401.374	1.003

Avg=(∑/total number of observations), Min=Minimum value in sample size, Max=Maximum value in sample, S. D=Standard Deviation, SEM={SD/SQRT(500)}, Range=Max-Min, Median=Median of all sample size, Sum=∑ of all sample size, Mean=∑ of sample size/500. Avg=(∑/total number of observations), Min=Minimum value in sample size, Max=Maximum value in sample, S. D=Standard Deviation, SEM={SD/SQRT(500)}, Range=Max-Min, Median=Median of all sample size, Sum=∑ of all sample size, Mean=∑ of sample size/500.

Table 6 showed that the values of morphometric parameters of shape of erythrocytes i.e., round are higher (0.930) in cattle followed by buffalo (0.914), then sheep (0.901) and goat respectively, the lowest was seen in the goat (0.893). Similarly, the values of solidity are equal in buffalo and cattle (1.003), while the value of solidity was lower but equal in goats and sheep, that is (1.002). All values were expressed in microns.

Table 6. Reference values of morphometric parameters (area, length, width, perimeter, height, ferret, roundness, and solidity) of size of erythrocytes in livestock. (All results expressed in microns) (N=400) (Sample smear=2000)

Reference values of livestock (Goat, Sheep, Cattle, and Buffalo)				
Parameter of size	Goat	Sheep	Cattle	Buffalo
(a)	7.81	9.67	17.76	16.45
(l)	1.64	3.17	4.76	4.27
(w)	3.16	3.51	4.75	4.55
(p)	9.69	10.90	14.89	14.36
(h)	3.00	3.43	4.72	4.58
(f)	3.26	3.65	4.91	4.78
(r)	0.89	0.90	0.93	0.91
(s)	1.002	1.002	1.003	1.003

Table 7 showed the typical shapes of erythrocytes for livestock are a biconcave disc or discoid. However, the change in the form of erythrocytes occurs in different morphological and physiological conditions of the hematological disturbances or anemia (Žaja *et al*., 2019).

Table 7. Shape of Erythrocytes (Roundness, Solidity) in livestock (goat, sheep, cattle and buffalo)

Species	Shape of Erythrocytes	Roundness	Solidity
Goat	Tear drop, spindle shape, acanthocytes discoid with central pallor, disc with no central pallor	0.893	1.002
Sheep	Acanthocytes, disc with central pallor, disc with no central pallor, crenated (echinocytes)	0.901	1.002
Cattle	Crenated (echinocytes) with central pallor biconcave,	0.93	1.003
Buffalo	Biconcave with no central pallor	0.914	1.003

DISCUSSION

According to (Adili *et al*., 2013), the erythrocytes of young are greater than those of the erythrocytes of adults. The size of the cell decreases from proerythroblast to erythrocyte during erythropoiesis. The increase in erythrocyte size seen in our observations could be explained by hypoxia, which causes a reduction in erythropoiesis time by passing through several stages of maturation, resulting in large erythrocytes. According to this study, the circumference, area, and diameter of erythrocytes appeared to have a significant influence on the species of the determination of domestic animals. Moreover, ovine erythrocytes are slightly more minor than many mammal's erythrocytes, they have a width of (3.2–5) μm, a diameter of (4.5) μm, and a life cycle of (70–150) days. Further, they do not collect or lose shaped as early as erythrocytes of other species. A very small difference in values of the cited morphometrical parameters related to the study (Adili *et al*., 2016, 2017), is expected to be correlated to the various breeds of sheep. The values of (round 0.901) μm and (solidity 1.002) μm are nearly equal to those of (Žaja *et al*., 2019), although sheep breeds are different. The value of area obtained in this study (9.670) μm does not relate with the results described by (Adili and Melizi, 2014), their results of the area of erythrocytes were (15_19) μm. Both rams and ewes were nearly equal, which is astonishing. In sheep results of the influence of age exactly matches to (Adili *et al*., 2013), that the parameters of erythrocytes in young are bigger than that of adults.

In cattle the results were conversely different, the erythrocytic size has no significant difference between the young and adult within the females, while a significant difference ($p<0.001$) was present in young and adult males; the results match with (Dash, 2020). According to (Adili *et al*., 2013), size of the erythrocyte is larger in males as compared to females, our results were the same as (Adili *et al*., 2013), so it may be considered a bovine property or linked trait in all these species of animals. As DWR is a hot, dry region, water and vegetation depends on rainfall

which is very low. Area of erythrocytes (16.424) μm, perimeter (14.344) μm, height (4.571) μm, length (4.275) μm, and width (4.552) μm was in cattle's erythrocytes. However, age affects the size of erythrocytes in other animals. The size of erythrocyte of young in buffalo is higher than adults. The stage at which erythrocytes are discharged into the circulation from the bone marrow influence their size (Manwani and Bieker, 2008). The cellular oxygen level regulates erythropoiesis and erythrocyte mass in normal animals. From pro-erythroblast to erythrocyte, cell size decreases during erythropoiesis (Manwani and Bieker, 2008). For the diameter of erythrocytes, it's vibrant to see that the results are consistent and always correlate to worldwide reference values cited by (Meinkoth, 2000) and (Rizzi and Clinkenbeard, 2010).

Furthermore, as there are almost no studies and published work on buffalo at all on all these parameters of the size of erythrocytes were found, detailed information on morphometric parameters of erythrocytes of buffalo, cattle, sheep, and goats. We further tried to establish a reference value for all those given in Table 6 by obtaining statistically interpretable results. In livestock of Cholistan (Goat, Sheep, Buffalo, and Cattle), various forms of erythrocytes were observed. They were either biconcave with no central pallor in buffalo or in other shapes. In the case of cattle, biconcave and crenated (echinocytes) types of erythrocytes were found. Echinocytes or crenated types of erythrocytes have evenly spaced spicules. Acanthocytes or spur cells of erythrocytes with irregular shapes in different sizes of spicules were found in goats. Dacrocytes or teardrop-shaped erythrocytes were also observed in all age groups of male and female goats. Spindle-shaped erythrocytes were also found in goats. Acanthocytes or disc-shaped erythrocytes were found in sheep, as seen in the (Dash, 2020), the morphological features and breed on the morphometry of erythrocytes of female cattle.

Statistical analysis was carried out with ANOVA in Table 6, which shows major significant differences between the three regions ($p \leq 0.9$). According to gained results, it looks like altitude can influence the size of erythrocytes in goats. This increase in the erythrocyte size parameters at higher sea levels shown in our results might be interpreted as hypoxia that may occur at higher sea levels. It may shorten the time it takes for erythropoiesis to occur by delaying some phases of maturation to produce larger erythrocytes. The stage at which erythrocytes are discharged into circulation from the bone marrow determines their size (Manwani and Bieker, 2008; Schalm, 1974) the cellular O_2 level regulates erythropoiesis and erythrocyte mass in normal animals (Grondin, 2010). From problast to erythrocyte, cell size decreases during erythropoiesis (Manwani and Bieker, 2008). Firstly, in (Fig. 1) an overlap was seen between the three regions.

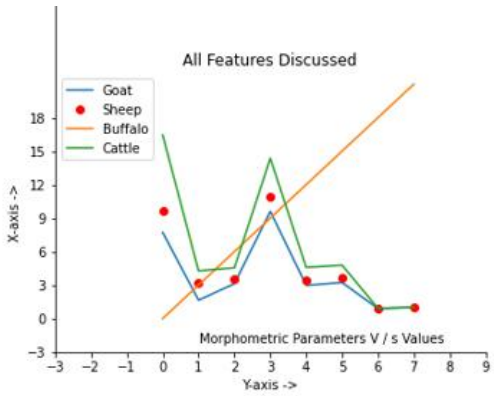

Figure 1. Python module of matplotlib is used to show the X-axis and Y-axis of the parameters of erythrocytes of different species being analyzed in this study.

Firstly, in (Fig. 1) an overlap was seen between the three regions. The altitude of KPT remained highest, whereas the size of erythrocyte in a parameter of area was lowest. While the altitude of DWR was lowest and size of erythrocyte was remained highest as compared to others. Secondly, YZN and KPT are closely connected to each other. The pattern remained same for goat and sheep, whereas some irregularities were observed in Cattle and Buffalo.

Furthermore, we must point out that traditional ocular micrometer measurements of erythrocyte size are difficult and imprecise. The above-mentioned software was used to measure the trails, allowing for more exact learning of comparisons in preventing the anthropoid feature intricated in the quantities understood with the ocular micrometer by selecting the top spot. In accumulation, this novel method of measurement

was very modest, shortest, easy to type, classy, and not very pricey.

CONCLUSION

A morphometric investigation of goat erythrocytes with a substantial impact on four hundred (400) blood sample smears based on altitude, gender and age revealed that erythrocytes are much larger in young animals than in adults. The morphometry of caprine erythrocytes was unaffected by gender, as was the case in the previous study (Adili *et al*., 2013). It appeared to be quite evident that the shape and size of the erythrocytes in sheep morphological characteristics are important for detecting variations in normal erythrocytic morphometry. Furthermore, it can be inferred that ovine erythrocytes may be investigated by computer-guided image analysis application of erythrocytes descriptive approaches, such as cluster analysis and principal component analysis, based on morphometric size and form criteria. However, the different physiological conditions may change the distribution of erythrocytes. Age and sex have a profound effect on the morphometry of erythrocytes of zebu cattle. Extended studies of different breeds of livestock are highly recommended. According to the findings, morphometric investigations of erythrocytes performed using advanced and reliable computer-guided software were more suitable and error-free than traditional morphometric approaches.

Based on our findings, we suggest that in the future there shall be more variation in the number of animals and samples. In our experiments, we defined twelve (12) categories for the number of males, females, young, and adults. These findings may be useful in the diagnosis of anemic illnesses in animals in veterinary medicine, notably in the cases of microcytic, normocytic, and macrocytic anemia. The shape of erythrocytes is significant in diagnosing anemia and other blood diseases. Smears of good quality with adequate Romanowsky/special staining, as well as morphologists' expertise, are still extremely valuable.

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