Figure 3: Crenated RBCs (echinocytes) are shown by (⟺) and spindle shaped RBC by (←).

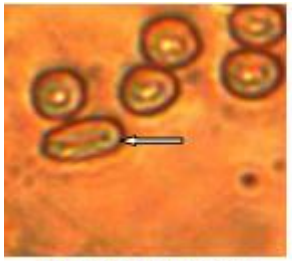

Figure 8: Leptocyte.

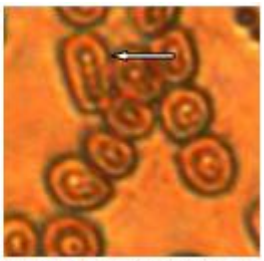

Figure 9: Stomatocyte.

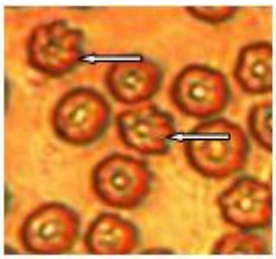

Figure 4: Acanthocytes.

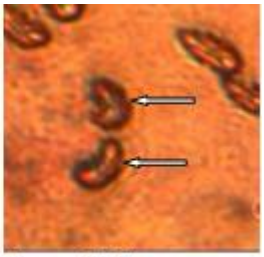

Figure 5: Comma shaped RBCs.

Figure 10: RBC with two central pallors.

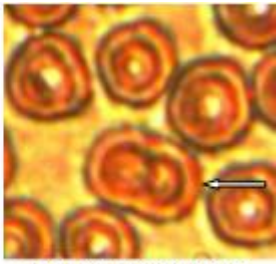

Figure 11: Dividing erythrocyte.

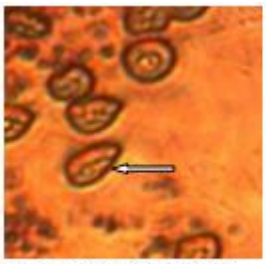

Figure 6: Dacryocyte (tear drop shaped RBC.

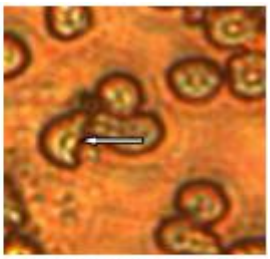

Figure 7: Schistocyte.

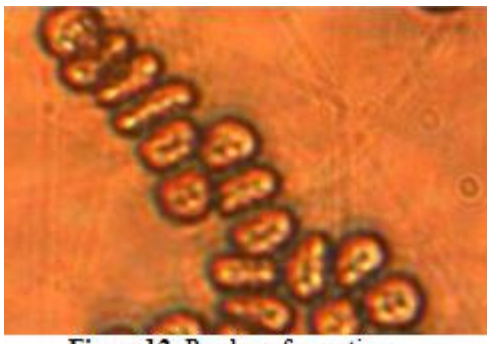

Figure 12: Rouleax formation.

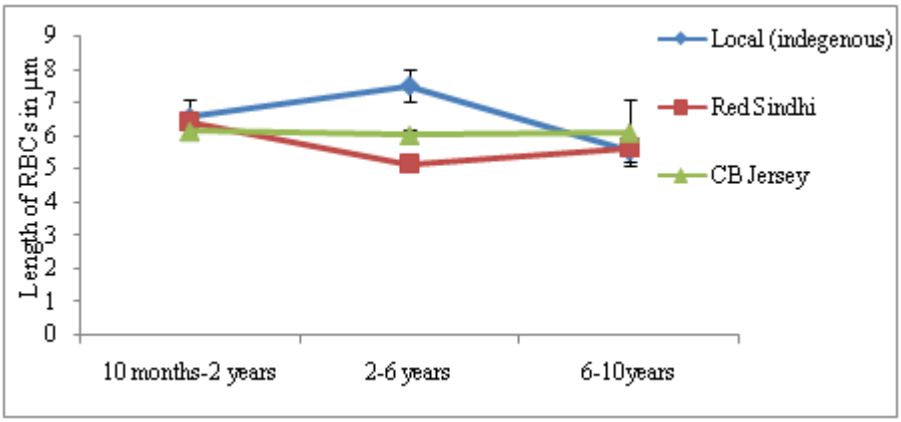

Figure 13: Influences of ages with respect to breeds on the length of RBCs. (CB Jersey, i.e., cross breed Jersey).

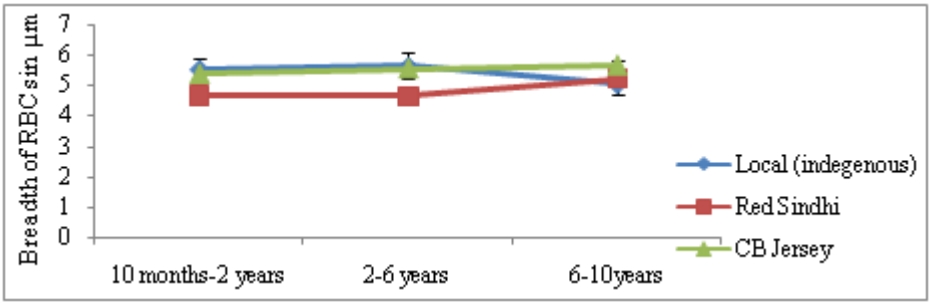

Figure 14: Influences of ages with respect to breeds on the breadth of RBCs. (CB Jersey, i.e., cross breed Jersey).

Table 1: Influences of age groups on the size of erythrocytes of three breeds of female cattle (Mean ± SE expressed in µm)

Types of breed	Parameter	Age groups			F value
		10months-2 years or Group1 (n=20)	2-6years or Group 2 (n=20)	6-10 years or Group 3 (n=20)	
Local (indigenous)	Length	6.56±0.50	7.51±0.48[b]	5.54±0.34[b]	4.75*
	Breadth	5.55±0.38	5.65±0.43	5.07±0.27	0.69 NS
Red Sindhi	Length	6.40±0.31[a]	5.15±0.21[a]	5.62±0.19	6.47**
	Breadth	4.68±0.30	4.66±0.23	5.23±0.21	1.61 NS
Cross breed Jersey	Length	6.13±0.14	6.01±0.17	6.09±0.09	0.20 NS
	Breadth	5.37±0.18	5.55±0.24	5.66±0.15	0.69 NS

[1]Mean±SE with similar superscripts in the same row differ significantly at p<0.05 and p<0.01
[2] * means Significant at p<0.05, ** means significant at p<0.01and NS means not significant
[3]Figures in parentheses represent the number of observations in each case
SE: Standard error, F value: Fischer's value

Table 2: Influences of breeds on the size of erythrocytes of different age groups of female cattle (Mean ± SE expressed in µm)

Age groups	Parameters	Breed			F value
		Local (indigenous)	Red Sindhi	Cross breed Jersey	
10 months-2 years (Group 1) (n=20)	Length	6.56±0.50	6.40±0.31	6.13±0.14	0.36 NS
	Breadth	5.55±0.38	4.68±0.30	5.37±0.18	2.31 NS
2-6 years (Group 2) (n=20)	Length	7.51±0.48[a]	5.15±0.21[a]	6.01±0.17[a]	13.59**
	Breadth	5.65±0.43	4.66±0.23	5.55±0.24	2.89 NS
6-10 years (Group 3) (n=20)	Length	5.54±0.34	5.62±0.19	6.09±0.09	1.56 NS
	Breadth	5.07±0.27	5.23±0.21	5.66±0.15	1.95 NS

[1]Mean±SE with similar superscripts in the same row differ significantly at p<0.01
[2] ** means significant at p<0.01and NS means not significant
[3]Figures in parentheses represent the number of observations in each case
SE: Standard error, F value: Fischer's value

3.2 Discussion

3.2.1 Morphology

Poikilocytosis is a general term used to describe the presence of erythrocytes having abnormal shape [4]. Poikilocytosis may be seen in clinically normal young cattle [22]. Echinocytes are spiculated erythrocytes having relatively evenly spaced and similar sized spicules [23]. When the surface area of the outer lipid monolayer increases relative to the inner monolayer echinocytosis form [4]. Acanthocytes or spur cells are erythrocytes with irregularly spaced, variably sized spicules [24]. When erythrocyte membranes contain excess cholesterol compared to phospholipids acanthocytes form [4]. Marked acanthocytosis is reported in young goats and some young cattle [22], [25]. Erythrocyte fragments with pointed extremities are called schistocytes. Erythrocyte fragmentation may appear when erythrocyte are forced to flow through altered vascular channels or exposed to turbulent blood flow [4]. Some leptocytes appear folded (Figure 8) [4]. Leptocytes may be seen in iron deficiency anemia [26]. Polychromatophilic erythrocytes may sometimes appear as leptocytes [4]. Dacryocytes are teardrop shaped erythrocytes with single pointed or elongated extremities (Figure 6) [4]. In iron-deficient ruminants, dacryocytes are common erythrocyte shape abnormalities [27]. Due to thick blood film preparations stomatocytes most often occur as artifacts [4].

3.2.2 Influence of age

Anisocytosis are seen in different age groups. According to some authors [28]-[30] age can be considered when establishing the references values in domestic animal. According to Schlam and Carlson [31], Harvey et al [28], Meinkoth and Clinkenbeard [32] and Harvey [1], the fetal erythrocytes are larger than those of adults. During gestation and at birth, the erythron compartment increase, at birth 9% of the red blood cells are reticulocytes [33]. Fetal calf red blood cells are less fragile and larger than adult bovine red blood cells [28]. The increasing of erythrocyte diameter with increase in age in group 1 cattle except local breed, observed in our study could be interpreted by the persistence of red blood cells after parturition formed during embryonic life and decreasing of the diameter or length by the stem cell adaptation to new conditions of life after parturition [34].

3.2.3 Influence of breed

Anisocytosis are seen in different breeds. Sex [35], breed [36], exercise [37], pregnancy and lactation [38]-[40], emotional states [15] are variables to be considered when establishing references values in domestic animal. Breed difference for both length and breadth is observed in our study which can be interpreted with some workers [15, 36] who had considered the breed as one of the factors for reference values. There is overlap between length of RBCs of local and Red Sindhi cattle among 10 months-2 years age (Figure 13). There is also found overlap between length of RBCs of 6-10 years of local and Red Sindhi cattle. There is slight overlap between breadth of 2-6 years of local and cross breed Jersey and between 6-10 years of local and Red Sindhi cattle (Figure 14).

4. Conclusion

Age and breed have effect on the morphometry of local (indigenous), Red Sindhi and cross breed Jersey female cattle and possible confusion of anemic syndromes can be avoided by this type of study. These results could serve as a base line for the diagnostic interpretation of anemic syndromes in veterinary medicine especially concerning normocytic, microcytic and macrocytic anemia. Extended studies to other breeds are highly recommended.

5. Acknowledgements

Authors would like to thank to the Head, P.G. Department of Zoology, Utkal University, Vani Vihar, Bhubaneswar, Odisha, India for providing them laboratory facilities to conduct this study. Thanks are due to Block veterinary officer and live stock inspector for providing blood samples.

References

[1] J.W. Harvey, "The erythrocyte: physiology metabolism and biochemical disorders," in Clinical Biochemistry of Domestic Animals, J.J. Kareko, J.W. Harvey and M.L. Bruss (eds.), 6th edn., San Diego: Academic Press, USA, pp. 173-240, 2008.
[2] J.W. Harvey, "Erythrocyte biochemistry," in Schlam's Veterinary Hematology, D.J. Weiss and K.J. Wardrop (eds.), 6th edn., Wiley-Blackwell Publishing Ltd., Ames. USA, Iowa, pp. 131-135, 2010.
[3] J.W. Kramer, "Normal hematology of cattle, sheep and goats," in Schlam's Veterinary Hematology, B.F. Feldman, J.G. Zinkl and N.C. Jain (eds.), 5th edn., Lippincott Williams and Wilkins, USA, Philadelphia, pp. 1075-1084, 2000.
[4] J.W. Harvey, Atlas of Veterinary Hematology: Blood and Bone marrow of Domestic Animals, WB Saunders Company, USA, Philadelphia, pp. 1-40, 2001.
[5] A.M. Barger, "Erythrocyte morphology," in Schalm's Veterinary Hematology, D. J. Weiss, K.J. Wardrop (eds.), Wiley-Blackwell Publishing Ltd., Ames. USA, Iowa, pp. 144-151, 2010.
[6] D. Wood and G.F. Quiroz-Rocha, "Normal hematology of cattle," in Schalm's Veterinary Hematology, D.J. Weiss and K.J. Wardrop (eds.), 6th edn., Wiley-Blackwell Publishing Ltd., Ames. USA, Iowa, pp. 829-835, 2010.
[7] C.S. Olver, G.A. Andrews, J.E. Smith and J.J. Kaneko, "Erythrocyte structure and function," in Schalm's Veterinary Hematology, D.J. Weiss and K.J. Wardrop (eds.), 6th edn., Wiley-Blackwell Publishing Ltd., Ames, USA, Iowa, pp. 123-130, 2010.
[8] M.A. Thrall, "Erythrocyte morphology," in Veterinary Hematology and Clinical Chemistry M.A. Thrall, G. Weiser, R.W. Allison and T.W. Campbell (eds.), 2nd edn., Wiley-Blackwell A John Wiley and Sons, Inc., Publication, USA, pp. 63, 2012.
[9] P.M. Kapale, D.G. Jagtap, D.M. Badukale and S.K. Sahatpure, "Haematological constituents of blood of Gaolao cattle," Vet World, 1(4), pp. 113-114, 2008.
[10] J.P.A. Baak, "The Principles and advances of quantitative pathology," Analyt Quant Cytol Histol, 9, pp. 89-95,1985.
[11] P.J. Van Diest and J.P.A. Baak, "Morphometry," in Comprehensive cytopathology, M. Bibbo (ed.), WB Saunders Company, Philadelphia, pp. 946-964, 1991.
[12] M. Oberholzer, H. Christen, R. Ettlin, M. Buser, M. Oestereicher and R. Gschwind, "Some fundamental aspects of morphometry in clinical pathology, demonstrated on a simple, multipurpose analysis system," Analyt Quant Cytol Histol, 13, pp. 316-320, 1991.
[13] R. Nafe, "Planimetry in pathology-a method in its own right besides stereology in automatic image analysis," Exp Pathol, 43, pp. 239-246, 1991.
[14] V. Russack, "Image cytometry: Current applications and future trends," Crit Rev Clin Lab Sci, 31, pp. 1-34, 1994.
[15] G.A. Sastry, Veterinary Clinical Pathology, 3rd edn., CBS Publishers and Distributors, Delhi, pp. 1-30, 1983.
[16] R.S. Brar, H.S. Sandhu and A. Singh, Veterinary Clinical Diagnosis by Laboratory Methods, 1st edn., Kalyani Publisher, Ludhiana, pp. 10, 2002.
[17] D. Ledieu, "Prélèvements encytologie," Dans: Encyclopédie vétérinarie, Editions Scientifiques et Médicales Elsevier, France, Biologie Clinique, 0030, 2003.
[18] W.J.J. Bacha and L.M. Bacha, Color atlas of veterinary, 2nd edn., Lippincott, Williams and Wilkins, USA, Philadelphia, pp. 27-36, 2000.
[19] D.B. Denicola, "Advances in hematology analyzers," Top Companion Anim M, 26(2), pp. 52-61, 2011.
[20] R.D. Lillie (ed.), HJ Conn's biological stains, 9th edn., The Williams and Wilkins Company, Baltimore, USA, pp. 606-607, 1977.
[21] N. Adili and M. Melizi, "Preliminary study of the influence of red blood cells morphometry on the species determinism of domestic animals," Vet World, 7 (4), pp. 219-223, 2014.
[22] T. Sato and M. Mizuno, "Poikilocytosis of newborn calves," Nippon Juigaku Zasshi, 44, pp. 801-805, 1982.
[23] D.J. Weiss, A. Kristensen, N. Papenfuss and C.B. McClay, "Quantitative evaluation of echinocytes in the dog," Vet Clin Pathol, 19, pp. 114-1111, 1990.
[24] M. Bessis, Living blood cells and their ultrastructure, Springer- Verlag, New York, NY, 1973.
[25] S.R. McGilivary, G.P. Searcy and V.M. Hirssch, "Serum iron, total iron binding capacity, plasma copper and hemoglobin types in anemic and poikilocytic calves," Can J Comp Med, 49, pp. 286-290, 1985.
[26] J.W. Harvey, "Microcytic anemias," in Scalm's Veterinary Hematology, B.F. Feldman, J.G. Zinkj and N.C. Jain (eds.), 5th edn., Lippincott Williams &Wilkins, Philadelphia, PA, pp. 200-204, 2000.
[27] D.E. Morin, F.B. Garry and M.G. Weiser, "Hematologic responses in llamas with experimentally-induced iron deficiency anemia," Vet Clin Pathol, 22, pp. 81-85, 1993.
[28] J.W. Harvey, R.L. Asquith, P.K. Mcnulty, J. Kivipelto and J.E. Bauer, "Hematology of the foals up to one year old," Equine Vet J, 16(4), pp. 347-353, 1984.
[29] H.E. Brun-Hansen, A.H. Kampen and A. Lund, "Hematologic values in calves during the first six months of life," Vet Clin Pathol, 35(2), pp. 182-187, 2006.
[30] T. Aoki and H. Ishii," Hematological and biochemical profiles in peripartum mares and neonatal foals (heavy Draft horse)," J Equine Vet Sci, 32, pp. 170-176, 2012.
[31] O.W. Schlam and G.P. Carlson, Equine Medicine and Surgery: the blood and the blood forming organs, 3rd edn., American veterinary publication, USA, 1982.
[32] J.H. Meinkoth and K.D. Clinkenbeard, "Normal hematology of the dog," in Schalm's Veterinary Hematology, B.F. Feldman, J.G. Zinkl and N.C. Jain (eds.), 5th edn., Lippincott Williams and Wilkins, USA, Philadelphia, pp. 1057-1063, 2000.
[33] K. McGrath and J. Palis, "Ontogeny of erythropoiesis in the mammalian embryo," Curr Top Dev Biol, 82, pp. 1-22, 2008.
[34] N. Adili, M. Melizi and O. Bennoune, "The influence of age, sex and altitude on the morphometry of red blood cells in bovines," Vet World, 6(8), pp. 476-478, 2013.

[35] A.H. Shaikat, M.M. Hassan, S.H. Khan, M.N. Islam, M.A. Hoque, M.S. Bari and M.E. Hossain "Hemato-biochemical profiles of indigenous goats (*Capra hircus*) at Chittagong, Bangladesh," Vet World, 6(10), pp. 789-793, 2013.
[36] M.C. Acena, S. Garcia-Belenguer, M. Garson M, and A. Purroy, "Modifications hematologiques at musculaires pendant la corrida chez le taureau de combat," Rev Méd Vét, 146 (4), pp. 277-282, 1995.
[37] R. Zobra, M. Ardu, S. Niccolini, F. Cubeddu, C. Dimauro, P. Bonelli, C. Dedola, S. Visco and M.L.P. Parpaglia, "Physical, hematological and biochemical responses to acute intense exercise in polo horses," J Equine Vet Sci, 31, pp. 542-548, 2011.
[38] F. Masoni, M. Lagadic, G. Plassiocrt, L.et Guigand and M. Wyers, "Paramèters Hématologiques de la chèvre laitière Variations physiologiques chez l' animal Sain autour de la mise-bas," Rec Méd Vét, 161(1), pp. 41-49, 1985.
[39] S. Roy, M. Roy and S. Mishra, "Hematological and biochemical profile during gestation period in Sahiwal cows," Vet World, 3(1), pp. 26-28, 2010.
[40] J. Mariella, A. Pirrone, F. Gentilini and C. Castagnetti, "Hematologic and biochemical profiles in standardbred mares during peripartum," Theriogenology, 81(4), pp. 526-534, 2014.

Authors Profile

Miss. Ipsita Dash has completed B.Sc. in Zoology in 2011 (first position in the University) from Fakir Mohan University, Vyasa Vihar, Balasore, Odisha, India. She has completed M. Sc. in Zoology in 2013 (third position in the University) and M.Phil. in Zoology (First position in the University) in 2014 from P.G. Department of Zoology, Utkal University, Vani Vihar, Bhubaneswar, Odisha, India.

Prof. Prafulla K. Mohanty is serving as a Professor and Head of the P.G. Department of Zoology, Utkal University, Vani Vihar, Bhubaneswar, Odisha, India. He has authored three research books, one monograph, one dictionary and 60 research papers. He has already guided 19 Ph.D. scholars and at present 08 Ph.D. scholars are undertaking research under his supervision.