



Assessment of age and sex variability on haematological parameters of indigenous Punganur cattle

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ABSTRACT

This study aimed to assess the variability of sex and age on the hematological parameters of Punganur cattle. Blood samples were collected from 175 healthy Punganur cattle from Livestock Research Station (LRS), Sri Venkateswara Veterinary University and from farmers in the surrounding areas of Palamaner, Chittoor district, Andhra Pradesh across various age groups (Cows, Bulls, Heifers, Young bulls, Female calves, and Male calves) were collected and analysed. The results were categorized based on sex and age variations. Lymphocyte percentage ($P < 0.01$) and granulocyte percentage ($P < 0.05$) exhibited statistically significant variations, however, all other parameters did not show any differences under sex. Age-based analysis revealed significant differences ($P < 0.01$) in Total Leucocyte Count Lymphocyte Count, Lymphocyte Percentage %, Granulocyte %, Total Erythrocyte Count, Haemoglobin, Mean Corpuscular Haemoglobin Concentration, Red Cell Distribution Width, Platelet Count, Platelet Distribution Width, and Plateletcrit, with Mean Corpuscular Volume showing significance at $P < 0.05$. The findings could be used as reference values to differentiate normal physiological, nutritional and disease conditions for females, males and different age groups of Punganur cattle. This provides valuable insights for veterinarians and farmers, contributing significantly to the conservation of this endangered breed.

Keywords: Haematology, Punganur, Variations based on sex, Variations based on age

India's cattle genetic resources are remarkably diverse, comprising 55 recognized breeds as of October 14, 2025, according to the ICAR-National Bureau of Animal Genetic Resources (NBAGR, 2025), with the Punganur breed assigned the accession number INDIA_CATTLE_0100_PUNGANUR_03022. The Punganur breed is distinguished by its diminutive size, with males typically weighing around 240 kg and females approximately 170 kg originating from the Chittoor district (Andhra Pradesh). These cattle are dual-purpose, utilized for both milk production and draught work. Despite their small stature, with an average daily milk yield of approximately 3 litres, Punganur cattle are particularly noted for their high milk fat content (Ekambaram *et al.* 2014). The breed has evolved to thrive in the region's challenging environmental conditions, characterized by low rainfall and frequent droughts, by maximizing efficiency with minimal inputs,

while maintaining relatively good milk production levels (Bharathi *et al.* 2023). However, the breed is currently at risk of extinction due to the increasing preference for exotic and crossbred cattle, which has led to the reduction of Punganur herds (Vinod *et al.* 2024).

Hematological parameters are important indicators of the physiological, nutritional, and health status of ruminants and are widely used in disease diagnosis and monitoring. These parameters are influenced by factors such as breed, age, sex, nutrition, management, and environmental conditions (Etim *et al.* 2014). Indigenous cattle breeds of India exhibit better adaptability and stress tolerance under tropical conditions, which is reflected in their hematological profile. Previous studies on indigenous breeds such as Malnadgidda, Deoni, Krishnavalley, Pulikulam, and Kangayam have reported significant variations in hematological parameters, highlighting the importance of establishing breed-specific reference values for accurate clinical interpretation (Sripad *et al.* 2014b, 2018a, 2018b; Srinivasan and Sathiamoorthy 2021; Karumalaisamy *et al.* 2022). However, studies on Punganur cattle are limited and mainly focus on seasonal or general hematological evaluation with smaller sample sizes (Naik *et al.* 2013). Therefore, establishing comprehensive baseline hematological values in Punganur cattle is essential for improving health assessment, disease diagnosis, and conservation strategies. Hence, the

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present study was undertaken to establish a baseline hematological profile of Punganur cattle and to assess age and sex related variations.

MATERIALS AND METHODS

The present investigation was conducted on Punganur cattle maintained at the Livestock Research Station (LRS), Sri Venkateswara Veterinary University and from farmers in the surrounding areas of Palamaner, Chittoor district, Andhra Pradesh. The mean environmental temperature ranged from 21-22°C (minimum) to 33-35°C (maximum), with relative humidity levels between 53-56%.

A total of 175 healthy Punganur cattle were selected for the study, grouped into six categories: Group I: Cows (n = 52, after first calving); Group II: Bulls (n = 35, 4 years and above); Group III: Heifers (n = 27, 1 to 3 years); Group IV: Young bulls (n = 24, 1 to 4 years); Group V: Female calves (n = 17, below 1 year); and Group VI: Male calves (n = 20, below 1 year) and Females (n = 96) and Males (n = 79). About, 5 ml of whole blood was collected from the jugular vein of each animal aseptically between 6:00 AM and 7:30 AM, using 5 ml K3E (EDTA) BD Vacutainer tubes. Immediately following collection, the samples were labelled and transported to the laboratory for hematological analysis, which was performed within 24 hours using a Mindray BC-2800 Auto Hematology Analyzer.

Blood samples were collected three times from the same animals during the early morning hours before feeding, with a 15-day interval between two subsequent collection, resulting in 525 samples (175 samples per collection). The hematological profile for 18 parameters for the 525 samples was analysed to determine sex and age-based variations. Statistical analysis was performed using SPSS software employing repeated measures ANOVA to assess the age-related differences, while an independent sample t-test was used to evaluate variations based on sex.

RESULTS AND DISCUSSION

The baseline hematological profile of Punganur cattle are presented in Table 1 and have been compared with the available literature on the same parameters in Punganur cattle by Naik *et al.* (2013), as well as in other breeds such as Malnadgidda cattle (Sripad *et al.* 2018a), Deoni cattle (Sripad *et al.* 2018b), Krishnavalley cattle (Sripad *et al.* 2014a) and Khillar cattle (Sripad *et al.* 2014b).

Variations based on sex: Hematological parameters did not exhibit significant differences between the two genders (Table 1). However, two parameters, lymphocyte percentage ($P < 0.01$) and granulocyte percentage ($P < 0.05$), and warrant further investigation to better understand the underlying physiological or environmental factors contributing to these differences. It can perhaps be attributed to haematological parameters being more influenced by age, breed, and health status rather than sex, as both male and female cattle have similar physiological needs and metabolic activities, particularly when exposed to similar environmental and nutritional conditions, show minimal

variations in most blood parameters and other influencing factors include management, stress, and disease (Nseabasi *et al.* 2014).

Total Leucocyte Count (TLC) values for females ($8.56 \pm 0.25 \times 10^3/\mu\text{l}$) and males ($8.95 \pm 0.28 \times 10^3/\mu\text{l}$) in Punganur cattle were comparatively lower than those reported for Malnadgidda, Deoni, Krishnavalley and Khillar cattle, which reflects significant variations in their immune profiles, likely influenced by environmental adaptations and breed-specific characteristics. The lymphocyte percentages for females and males in Punganur cattle were $51.32 \pm 0.90\%$ and $55.18 \pm 1.00\%$, respectively, which were lower than the values reported for Krishnavalley and Khillar cattle. Conversely, the monocyte percentages for Punganur cattle were comparatively higher than those observed in Krishnavalley and Khillar cattle.

The Total Erythrocyte Count (TEC) values observed for Punganur cattle were $6.74 \pm 0.13 \times 10^6/\mu\text{l}$ for females and $6.72 \pm 0.14 \times 10^6/\mu\text{l}$ for males and were comparable to those reported for Deoni cattle but lower than those recorded for Malnadgidda and Krishnavalley cattle. The mean haemoglobin (Hb) concentrations for females (9.16 ± 0.13 g/dl) and males (9.09 ± 0.14 g/dl) were in a similar trend with Krishnavalley cattle, however higher than those for Malnadgidda cattle and lower than Deoni cattle. The Haematocrit (HCT) or Packed Cell Volume (PCV) percentages observed in Punganur females ($27.70 \pm 0.44\%$) and males ($27.27 \pm 0.49\%$) were lower than Malnadgidda, Deoni, Krishnavalley and Khillar cattle.

The Mean Corpuscular Volume (MCV) values for

Table 1. Hematological Profile of Female and Male Punganur Cattle (Mean \pm SE)

S. No.	Parameter	Female (n=96)	Male (n=79)
1	TLC ($10^3/\mu\text{l}$)	8.56 \pm 0.25	8.95 \pm 0.28
2	LYM ($10^3/\mu\text{l}$)	4.47 \pm 0.17	4.97 \pm 0.19
3	MON ($10^3/\mu\text{l}$)	0.93 \pm 0.03	0.90 \pm 0.03
4	GRAN ($10^3/\mu\text{l}$)	3.16 \pm 0.09	3.01 \pm 0.11
5	LYM (%) **	51.32 \pm 0.90	55.18 \pm 1.00
6	MON (%)	11.00 \pm 1.85	13.49 \pm 2.06
7	GRAN (%) *	37.37 \pm 0.80	34.35 \pm 0.89
8	TEC ($10^6/\mu\text{l}$)	6.74 \pm 0.13	6.72 \pm 0.14
9	Hb (g/dl)	9.16 \pm 0.13	9.09 \pm 0.14
10	HCT/PCV (%)	27.70 \pm 0.44	27.27 \pm 0.49
11	MCV (fl)	41.93 \pm 1.76	43.63 \pm 1.96
12	MCH (pg)	13.85 \pm 0.64	14.75 \pm 0.71
13	MCHC (g/dl)	33.23 \pm 0.53	34.05 \pm 0.59
14	RDW (%)	17.87 \pm 0.17	18.19 \pm 0.19
15	PLT ($10^3/\mu\text{l}$)	287.86 \pm 12.51	308.00 \pm 13.88
16	MPV (fl)	5.76 \pm 0.23	5.98 \pm 0.26
17	PDW (%)	16.43 \pm 0.06	16.33 \pm 0.06
18	PCT (%)	0.22 \pm 0.04	0.17 \pm 0.05

** $P < 0.01$: Highly Significant * $P < 0.05$: Significant

Punganur cattle were lower than those observed in Malnadgidda, Deoni, Krishnavalley and Khillar cattle. While the Mean Corpuscular Haemoglobin (MCH) values were comparable to those of Krishnavalley and Khillar cattle, lower than those in Deoni cattle and higher than the Malnadgidda. The Mean Corpuscular Haemoglobin Concentration (MCHC) in Punganur cattle for both females and males was comparatively higher than that observed in Malnadgidda, Deoni, Krishnavalley and Khillar cattle. Regarding platelet count, the mean values for female Punganur cattle were higher than those of Malnadgidda, Deoni, Krishnavalley and Khillar cattle. For male Punganur cattle, the platelet count was also higher than that of Malnadgidda, Deoni and Krishnavalley breeds, while being in agreement with Khillar cattle. The mean values for Red Cell Distribution Width (RDW), Mean Platelet Volume (MPV), Platelet Distribution Width (PDW), and Plateletcrit (PCT) in Punganur cattle could not be compared with other breeds due to a lack of available data regarding these parameters.

The mean values of hematological parameters across different age groups are presented in Table 2. Significant differences ($P < 0.01$) were observed in TLC, LYM, Lymphocyte Percentage, Granulocyte Percentage, TEC, Hb, MCHC, RDW, PLT, PDW, and PCT. Additionally, a significant difference ($P < 0.05$) was noted in MCV

among the six age groups of Punganur cattle. However, no significant differences were observed in MONO and HCT or PCV, MCH, and MPV among the various age groups.

TLC was in the range of 7.95 ± 0.33 to 10.53 ± 0.53 thousands/ μ l with lower values in the cows and higher values recorded in male calves. The range falls within the reported values by Naik *et al.* (2014) in Punganur, however, they reported higher TLC values in adult animals than in younger animals which was contrary to our findings. These differences might have resulted from lack of immune system maturation in younger animals, causing higher leukocyte counts as part of their active immune development (Khan *et al.* 2018). The mean lymphocyte count ranged from 3.90 ± 0.21 (cows) to 6.25 ± 0.34 (male calves), with lymphocyte percentages ranging between 48.80 ± 1.16 % in cows and 58.63 ± 2.02 % in female calves. These results are consistent with the range reported by Naik *et al.* (2014) in Punganur cattle. However, Naik *et al.* found higher lymphocyte percentages in adults compared to younger animals, which contrasts with our findings. This suggests younger animals often exhibit higher lymphocyte counts as part of their developing immune systems (Karumalaisamy *et al.* 2022) and this immune response tends to be more active in calves compared to adult cattle.

The monocyte % of our study was reported to be higher than the findings of Naik *et al.* (2014) who reported

Table 2. Hematological profile of Punganur cattle at different age groups (Mean \pm SE)

Age Groups	TLC ($10^3/\mu$ l) **	LYM ($10^3/\mu$ l) **	MON ($10^3/\mu$ l) ^{NS}	GRAN ($10^3/\mu$ l) ^{NS}	LYM (%) **	MON (%) ^{NS}
Cows	7.95 ± 0.33 ^a	3.90 ± 0.21 ^a	0.89 ± 0.04	3.16 ± 0.13	48.80 ± 1.16 ^a	11.21 ± 2.52
Bulls	8.12 ± 0.41 ^a	4.36 ± 0.26 ^a	0.86 ± 0.05	2.73 ± 0.16	54.73 ± 1.43 ^{ab}	17.81 ± 3.12
Heifers	8.98 ± 0.46 ^{ab}	4.79 ± 0.30 ^a	0.98 ± 0.05	3.22 ± 0.18	51.54 ± 1.60 ^{bc}	11.04 ± 3.50
Young bulls	8.81 ± 0.49 ^{ab}	4.75 ± 0.31 ^a	0.91 ± 0.06	3.14 ± 0.19	52.99 ± 1.70 ^c	10.87 ± 3.71
Female calves	9.75 ± 0.58 ^{bc}	5.70 ± 0.37 ^b	0.99 ± 0.07	3.07 ± 0.22	58.63 ± 2.02 ^{ab}	10.26 ± 4.41
Male calves	10.53 ± 0.53 ^c	6.25 ± 0.34 ^b	0.97 ± 0.06	3.31 ± 0.21	58.54 ± 1.87 ^c	9.27 ± 4.06
Age Groups	GRAN (%) **	TEC ($10^6/\mu$ l) **	Hb (g/dl) **	HCT/PCV (%) ^{NS}	MCV (fl) *	MCH (pg) ^{NS}
Cows	39.93 ± 1.03 ^c	6.49 ± 0.14 ^a	9.28 ± 0.16 ^{bc}	28.26 ± 0.59	43.87 ± 2.34 ^{ab}	14.40 ± 0.86
Bulls	34.45 ± 1.27 ^{bc}	6.39 ± 0.18 ^a	9.28 ± 0.20 ^{ab}	28.65 ± 0.73	43.07 ± 2.90 ^{ab}	14.22 ± 1.07
Heifers	36.26 ± 1.43 ^{ab}	6.36 ± 0.20 ^a	8.68 ± 0.23 ^{bc}	26.78 ± 0.82	42.67 ± 3.25 ^{ab}	13.74 ± 1.20
Young bulls	36.11 ± 1.52 ^a	6.30 ± 0.22 ^b	8.43 ± 0.24 ^c	25.76 ± 0.87	50.48 ± 3.45 ^a	16.60 ± 1.27
Female calves	31.31 ± 1.80 ^{bc}	8.09 ± 0.26 ^a	9.56 ± 0.28 ^a	27.43 ± 1.03	34.83 ± 4.10 ^b	12.38 ± 1.51
Male calves	32.03 ± 1.66 ^{ab}	7.78 ± 0.24 ^b	9.55 ± 0.26 ^c	26.74 ± 0.95	36.36 ± 3.78 ^a	13.43 ± 1.39
Age Groups	GRAN (%) **	TEC ($10^6/\mu$ l) **	Hb (g/dl) **	HCT/PCV (%) ^{NS}	MCV (fl) *	MCH (pg) ^{NS}
Cows	39.93 ± 1.03 ^c	6.49 ± 0.14 ^a	9.28 ± 0.16 ^{bc}	28.26 ± 0.59	43.87 ± 2.34 ^{ab}	14.40 ± 0.86
Bulls	34.45 ± 1.27 ^{bc}	6.39 ± 0.18 ^a	9.28 ± 0.20 ^{ab}	28.65 ± 0.73	43.07 ± 2.90 ^{ab}	14.22 ± 1.07
Heifers	36.26 ± 1.43 ^{ab}	6.36 ± 0.20 ^a	8.68 ± 0.23 ^{bc}	26.78 ± 0.82	42.67 ± 3.25 ^{ab}	13.74 ± 1.20
Young bulls	36.11 ± 1.52 ^a	6.30 ± 0.22 ^b	8.43 ± 0.24 ^c	25.76 ± 0.87	50.48 ± 3.45 ^a	16.60 ± 1.27
Female calves	31.31 ± 1.80 ^{bc}	8.09 ± 0.26 ^a	9.56 ± 0.28 ^a	27.43 ± 1.03	34.83 ± 4.10 ^b	12.38 ± 1.51
Male calves	32.03 ± 1.66 ^{ab}	7.78 ± 0.24 ^b	9.55 ± 0.26 ^c	26.74 ± 0.95	36.36 ± 3.78 ^a	13.43 ± 1.39

** P < 0.01: Highly Significant *P < 0.05: Significant. Means with similar superscripts within a column do not differ significantly

a monocyte % between 6.45 ± 0.22 % to 6.98 ± 0.21 %, with adults having a higher percentage of monocytes than young animals. Monocyte percentages can vary significantly between age groups as seen in transcriptional activity and pathogen response (Wang *et al.* 2023) and monocyte numbers are influenced by genetic factors and environmental conditions (Vlasova *et al.* 2021). No significant variations were observed in granulocyte counts, which ranged from $2.73 \pm 0.16 \times 10^3/\mu\text{l}$ to $3.31 \pm 0.21 \times 10^3/\mu\text{l}$. However, granulocyte percentages differed, with values from 31.31 ± 1.80 % to 39.93 ± 1.03 %. Female calves had lower values and higher values were seen in cows, and adult cattle exhibited higher granulocyte percentages than calves. These trends highlight age- and gender-related differences in granulocyte percentages while younger calves often show lower values due to their developing immune responses (Villa *et al.* 2021).

The mean TEC for different age groups of Punganur cattle ranged from $6.30 \pm 0.22 \times 10^6/\mu\text{l}$ to $8.09 \pm 0.26 \times 10^6/\mu\text{l}$, with lower values observed in young bulls and higher values in female calves. This range falls below the reported values by Naik *et al.* (2014) for TEC in Punganur cattle, and our findings do not align with their conclusions, as they reported higher values in bulls than in other age groups. This discrepancy may be attributed to significant variations in TEC values influenced by seasonal changes, age, and stress factors (Farooq *et al.* 2023).

The mean haemoglobin values in our study ranged from 8.43 ± 0.24 g/dl in young bulls to 9.56 ± 0.28 g/dl in female calves, which fall below the range reported by Naik *et al.* (2014) for Punganur cattle, who documented higher haemoglobin concentrations in male calves and lower levels in young bulls, consistent with our findings. Studies on Pulikulam cattle by Srinivasan *et al.* (2021) also reflect age-based variations, showing higher haemoglobin levels in calves compared to adults. Additionally, research by Wyk *et al.* (2013) on East African short-horn zebu calves reported that haemoglobin levels tend to increase during the first few months of life, peaking around 42 days, and then gradually decreasing as the animals mature.

The mean PCV or Haematocrit in our study ranged from 25.76 ± 0.87 % to 28.65 ± 0.73 % across different age groups, with lower values in young bulls and higher values in bulls. These values were lower than the range typically reported for Punganur cattle by Naik *et al.* (2014), yet in line with their observations of higher values in adults compared to calves. This suggests that PCV values across different age groups may be influenced by factors such as age, nutrition, hydration status, disease, and environmental conditions. Lower PCV values are also commonly observed in younger animals due to their developing physiology and higher metabolic demands (Turkson *et al.* 2015).

The mean MCV values in our study ranged between 34.83 ± 4.10 fl and 50.48 ± 3.45 fl, with lower values in female calves and higher values in young bulls and adults, indicating significantly higher values than those observed in calves. The mean MCH values ranged from 12.38 ± 1.51 pg

to 16.60 ± 1.27 pg, following the MCV trend and showing no significant differences among the different age groups. The mean values for MCHC in the present study ranged from 32.42 ± 0.97 g/dl to 37.66 ± 1.12 g/dl, with lower values in heifers and higher values in male calves. This contrasts with the patterns observed for MCV and MCH. The relationship between MCHC, MCV, and MCH is commonly observed, reflecting the various maturation stages of red blood cells, with younger animals often exhibiting more immature and compact red cells, resulting in elevated MCHC values (Li *et al.* 2024). Younger animals, such as calves, display elevated MCHC compared to adults due to physiological differences in red blood cell production (eClinPath). These findings support the notion that hematological parameters, including MCHC, MCV, and MCH, vary not only with age but also in accordance with the developmental needs and health status of the animal. The RDW (%) in Punganur cattle ranged from 17.32 ± 0.21 % to 19.38 ± 0.34 %, with lower values in cows and higher values in male calves. The elevated RDW percentage in calves suggested anisocytosis, which is a common finding in younger animals due to active erythropoiesis and the release of immature red blood cells into circulation (Juan-Moreo *et al.* 2019).

In our study, the mean platelet counts ranged from $254.16 \pm 22.24 \times 10^3/\mu\text{l}$ to $409.37 \pm 28.03 \times 10^3/\mu\text{l}$ across different age groups of Punganur cattle, with significantly lower values in heifers and higher values in female calves. This aligns with the findings of Allah *et al.* (2015), which indicated that younger animals often exhibit elevated platelet counts due to their rapid growth phase. No significant differences were observed in MPV (fl) among the different age groups, with values ranging from 5.05 ± 0.56 fl to 6.62 ± 0.51 fl in female and male calves, respectively. The mean PDW (%) values in Punganur cattle across different age groups ranged from 15.86 ± 0.12 % to 16.60 ± 0.09 %, with lower values in female calves and higher values in heifers. This pattern is consistent with the findings of González *et al.* (2023), which reported that PDW tends to be lower in younger animals due to their more uniform platelet sizes, reflecting the relative stability of platelet production during early life stages. In adults, variations in PDW may increase due to ageing or other physiological changes affecting platelet production and activation. The mean PCT (%) values ranged from 0.15 ± 0.08 % to 0.26 ± 0.06 %, showing lower values in heifers and higher values in cows, with no significant differences among the different age groups, suggesting that PCT tends to exhibit minimal variation across age (Allah *et al.* 2015).

This study provides a baseline hematological profile of Punganur cattle, emphasizing significant variability based on sex and age. While gender related differences were insignificant, age-related variability was more pronounced, with parameters such as total leucocyte count, erythrocyte count, hemoglobin concentration, and platelet count showing significant differences across age groups. Younger animals, particularly calves, exhibited higher immune cell counts and platelet levels, reflecting their

active immune development, while adults demonstrated more stable hematological values. These findings underscore the importance of considering both sex and age when interpreting hematological data in Punganur cattle, as these factors play a crucial role in influencing physiological responses. Establishing these baseline values is vital for accurate health assessments and for designing targeted management and conservation strategies. Further research needs to focus on understanding the underlying physiological mechanisms driving these variations, contributing to improved breeding practices and conservation efforts for this indigenous breed.

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