RESEARCH ARTICLE

Hematological Profile of Indigenous Kangayam Calves in Relation to Age and Sex

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Abstract

The objective of the study was to establish the reference range of hematological parameters for indigenous Kangayam calves of Tamil Nadu, India. The indigenous male and female Kangayam calves from birth to one year of age of both sex were selected for this study (n = 10). Blood samples were collected at 3, 6, 9, and 12 months of age. The hematological parameters were estimated by an automated blood cell analyzer, and the morphometry of red blood cells was studied using an ocular micrometer in a microscope. The overall value (Mean ±SE) of hemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC), lymphocytes, monocytes, neutrophils, basophils, eosinophils, and platelet were recorded. Hb, PCV, and RBC values showed a highly significant ($p \leq 0.01$) difference in female Kangayam calves among different age groups among all hematological parameters. A significant ($p \leq 0.05$) decrease was recorded in WBC with advancement of age in Kangayam male calves. No significant difference was noticed in platelets and basophils in both sexes up to one-year age. Other parameters like lymphocyte, monocytes, neutrophils, eosinophils, MCV, MCH, and MCHC showed highly significant ($p \leq 0.01$) differences among the different age groups of both sexes. There is no significant difference in RBC size among the different age groups in both the sexes. The estimated hematological profile of Kangayam calves will serve as baseline values to the clinician during different clinical conditions.

Keywords: Age-wise, Blood profile, Hematological parameters, Kangayam calves, Sex wise.

Ind J Vet Sci and Biotech (2022): 10.21887/ijvsbt.18.2.9

Introduction

Indigenous breeds in India are well adopted locally with several characters like disease resistance, low input management, better survivability, and suitability for draught works (Kandasamy. 2001). There are 43 recognized cattle breeds in India (NBAGR, 2018). Among indigenous breeds of India, the Kangayam breed of cattle is one of the best-known superior draught breeds of Tamil Nadu. It is well known for its superior qualities, adaptation to poor nutrition, and longevity (Kandasamy. 2001).

The hematological values of calves during the early growth period are important to diagnose different clinical conditions. Moreover, the hematological values are likely to differ depending on age, sex, breed, geographic location, climatic variation, nutrition, husbandry practices, and analysis method. The young calves are more prone to bacterial and parasitic infections during the early growth period, leading to alteration of blood picture (Jain. 1986). The reference range for hematological and morphometric values is available only for adult Kangayam, and other indigenous animals (Lankesh *et al*., 2015; Kumar *et al*., 2017; Rajamanikam *et al*., 2018; Mili *et al*., 2020), and very scanty data are available for young calves (Mohri *et al*., 2007; Kim *et al*., 2012; Panousis *et al*., 2018). Hence, the present study was conducted to overcome the above lacunae by establishing a reference range of hematological profiles in young Kangayam calves of Tamil Nadu, India, relating to age and sex.

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How to cite this article: Karumalaisamy, D., Palanisamy, S., Santhanam, J., Balasundaram, K., Sivakumar, K., & Sakthivelu, M. (2022). Hematological Profile of Indigenous Kangayam Calves in Relation to Age and Sex. Ind J Vet Sci and Biotech. 18(2), 44-48.

Source of support: Nil

Conflict of interest: None.

Submitted: 22/05/2021 **Accepted:** 24/10/2021 **Published:** 10/04/2022

Materials and Methods

The study was conducted during the period from November 2018 to December 2019 on indigenous Kangayam calves at Kangayam Cattle Research Station, Sathyamangalam, and Erode district. A total of 80 animals (40 males and 40 females) were included in the present study. The blood samples were collected by venipuncture from the jugular vein at 3, 6, 9, and 12 months of age group (n=10 in each group) from both male and female Kangayam calves in the K3 EDTA containing tubes. The samples were obtained and analyzed on the

same day. However, samples were stored at 2-8°C while transferring to the test laboratory. A portion of blood was directly used to estimate red blood cell (RBC), hemoglobin (Hb), hematocrit (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT) and white blood cells count (WBC).

These parameters were measured in a hematology analyzer (VetScan HM5 hematology analyzer) provided with software for cattle. The percentage distribution of differential leucocytes was determined after smearing the peripheral blood and staining with Leishman's stain. The cells were counted manually and expressed in percentage. All hematological examinations were performed within 24 hours of sampling to avoid errors due to cell lysis.

For measurement of RBC size, the blood smear sample of different age groups was stained with Giemsa. The RBCs were measured using an ocular and objective micrometer under 100 X with standardization of ocular micrometer. This magnification was calibrated using a stage micrometer.

Statistical Analysis

The data collected were analyzed using one-way ANOVA (one-way analysis of variance) in SPSS Version 21.0 software package. Results are presented as means with a standard error, and the significant difference was considered at $p \leq 0.05$ and 0.01.

Results and Discussion

The blood profile of any cattle breed provides an opportunity to appraise the physiological, nutritional, and pathological status of individual breeds in diagnosis.

The values of hematological parameters like Hb, PCV, RBC, WBC, and percentage of different leucocytes, platelets, MCV,

Table 1: Mean (±SE) of hematological parameters of male and female Kangayam calves from 3-12 months of age

		Age in months			
Parameters	*Sex*	*3*	*6*	*9*	*12*
Hb (g/dL)	Male	11.54 ± 0.66	9.92 ± 0.34	10.50 ± 0.15	11.78 ± 1.24
	Female	9.97^{a} ± 0.12	10.81^{ab} ± 0.25	11.15^{b} ± 0.40	10.03^{a} ± 0.17
PCV (%)	Male	30.16 ± 1.61	28.15 ± 0.74	27.47 ± 3.11	32.97 ± 0.68
	Female	29.31^{a} ± 0.46	29.55^{a} ± 0.56	34.28^{b} ± 0.61	28.15^{a} ± 0.36
RBC(x10^{6}/µL)	Male	10.40 ± 0.47	9.01 ± 0.41	9.08 ± 0.40	9.94 ± 0.20
	Female	9.17^{b} ± 0.18	10.44^{c} ± 0.28	9.07^{b}± 0.13	8.25^{a}± 0.19
WBC(x10^{3}/µL)	Male	12.06^{b} ± 0.80	10.90^{ab} ± 0.62	9.86^{a} ± 0.18	11.81^{ab} ± 0.29
	Female	10.75 ± 0.18	11.23 ± 0.34	10.21 ± 0.18	10.28 ± 0.56
Lymphocyte (%)	Male	60.80^{a} ± 1.24	64.20^{a} ± 0.47	75.30^{b} ± 2.26	76.40^{b} ± 0.76
	Female	63.01^{a} ± 1.33	64.00^{a} ± 0.63	70.80^{b} ± 1.47	78.90^{c} ± 1.28
Monocyte (%)	Male	2.00^{a} ± 0.37	8.00^{b} ± 0.26	2.60^{a} ± 0.76	2.80^{a} ± 0.25
	Female	3.80^{b} ± 0.36	6.00^{c} ± 0.33	5.50^{c} ± 0.27	1.8^{a} ± 0.20
Neutrophils (%)	Male	32.60^{d} ± 0.60	27.20^{c} ± 0.59	19.10^{b} ± 2.40	10.80^{a} ± 0.90
	Female	30.08^{d} ± 1.35	26.90^{c} ± 0.72	19.40^{b} ± 1.60	14.0^{a} ±1.28
Basophils (%)	Male	0.60 ± 0.16	0.50 ± 0.17	0.70 ± 0.15	0.80 ± 0.20
	Female	0.60 ± 0.16	0.70 ± 0.21	0.60 ± 0.16	0.50 ± 0.17
Eosinophils (%)	Male	2.60^{a} ± 0.22	2.30^{a} ± 0.21	3.30^{a} ± 0.40	5.00^{b} ± 0.26
	Female	2.70^{a} ± 0.26	2.20^{a} ± 0.20	3.00^{a} ± 0.25	4.60^{b} ± 0.31
Platelet(x 10^{3}/µL)	Male	304.300 ± 8.08	312.40 ± 24.82	287.80 ± 14.05	301 ± 45.79
	Female	322.10 ± 13.85	318.70 ± 15.50	324.90 ± 21.48	371 ± 16.50
MCV (fl)	Male	35.20^{a} ± 0.65	35.60^{a} ± 0.45	37.10^{b} ± 0.86	37.40^{b} ± 0.62
	Female	35.50^{a} ± 0.70	35.40^{a} ± 0.37	36.20^{b} ± 0.23	36.20^{b} ± 0.14
MCH (pg)	Male	11.01^{a} ± 0.26	10.55^{a} ± 0.24	12.03^{b} ± 0.08	12.03^{b} ± 0.08
	Female	10.88^{a} ± 0.16	11.08^{a} ± 0.07	13.07^{b} ± 0.23	12.03^{b} ± 0.23
MCHC (%)	Male	30.28^{a} ± 0.47	31.39^{b} ± 0.52	31.95^{b} ± 0.44	32.95^{c} ± 0.64
	Female	30.14^{a} ± 0.18	30.40^{a} ± 0.30	31.23^{b} ± 0.37	31.23^{b} ± 0.21
RBC size (µm)	Male	5.80 ± 0.11	5.78 ± 0.10	5.23 ± 0.14	5.20± 0.25
	Female	5.85 ± 0.09	5.81 ± 0.14	5.38 ± 0.10	5.34 ± 0.09

** - Highly significant ($p < 0.01$), * - Significant ($p < 0.05$), ^{NS} - Non- significant ($p > 0.05$)
Means bearing different superscripts in a row differ significantly between groups.

MCH, and MCHC are presented in Table 1. Highly significant ($p \leq 0.01$) difference was observed in Hb, PCV, RBC values of female Kangaym calves among different age groups. A significant ($P \leq 0.05$) difference was recorded in WBC and MCHC among other groups in male calves. There is no significant difference noticed in platelet and percentage of basophils in both sexes during the growth phase of calves. Other variables significantly differed among the age group of both male and female Kangayam calves.

The value of Hb was higher during the early phase of growth, followed by a shortfall during 6rd and 9th months of age, then reached to 11.78 g/dL as adult range at 12 months in male Kangayam calves. A significant ($p \leq 0.05$) difference was noticed in female calves among age groups. The value of Hb was lower during the 3rd and 12th months, and a higher value was noticed during the 6th and 9th months. The lower value of Hb during the early phase of growth concurs with the reports of Mahima *et al.* (2013) and Kumar *et al.* (2017) in Hariana heifers and Sahiwal calves, respectively. The value of Hb in the present study was similar to the values reported by Jain. (1986) in 3-16 weeks old calves (11.2 ± 1), whereas higher Hb values (13-14) were observed by Kalyani *et al*. (2018) and Rajamanikam *et al*. (2018) in adult Kangayam cattle. This increasing trend of Hb in females during the early growth phase might be due to the shift of fetal hemoglobin to adult hemoglobin during early neonatal life (Jain. 1986).

The PCV values were significantly ($p \leq 0.01$) highest during the 9th month (34.28±0.61) of the growth phase. Kumar *et al*. (2017) reported a similar range of values in Sahiwal and Hardhenu calves of 0-1 year of age. The declining trend of PCV in early life could be due to probable expansion of plasma volume from milk consumption and increased destruction of fetal erythrocytes during the early life of the calves. The reported PCV of less than 24% is to be a sign of the anemic condition. Still, the Kangayam cattle calves did not show any anemic sign with lowered PCV percent to link the anemic sign reported by Marcotty *et al*. (2008). This could also be one reason that makes Kangayam cattle more suitable for a healthy lifespan (*Volk et al.,* 2019).

The numbers of RBC were found to be highest during the 3rd month in male Kangayam calves (10.40 ± 0.47) and significantly ($p \leq 0.01$) higher during the 6th month in female Kangayam calves (10.44 ± 0.28) and then starts to decline as that of adult in the latter phase of growth. The same increasing trend was observed by Mohri *et al.* (2007) during the first 3 months of age in Holstein's calves. In contrast, Kumar *et al.* (2017) reported a lower range of values (8.27 to 8.89) in Sahiwal and Hardhenu calves of 0-1 year of age compared to our results. The stress/excitement caused by venipuncture while collecting blood might be the cause of a slight increase in RBCs during the early months of growth (Jain. 1986).

The WBC counts were highest during the 3rd month in male Kangayam calves (12.06 ± 0.80). This value concurs with the findings of Kumar *et al*. (2017), who observed similar values of WBC in Hariana heifer (12.88), while a lower range of values (8.89-9.93) was observed in Sahiwal and Hardhenu calves of 0-1 year of age. The increasing level of leukocytes in the circulation may be due to a higher level of cortisol in the blood during the early life of the calves. It is also reported that WBC count was found to be comparable between male and female calves after six months of age (Mohri *et al*., 2007), which agrees with the current finding. The elevated levels of WBC in younger groups of male and female groups at three months of age could generally be attributed to some disease challenge with higher level of cortisol in the blood during the early life of the calves (Jain. 1986). However, there were no obvious clinical signs of disease in the calves when they were sampled during the study.

The increasing percentage of lymphocytes was noticed in both male and female Kangayam calves from 3 to 12 months (60-78 %) of age. Kumar *et al.* (2017) observed a similar range of values in Hardhenu, Sahiwal, and Hariana calves (61.33- 64.50 %) during 0-1 year of age. This may be due to falling neutrophil counts as a physiological phenomenon while aging advances which causes an increasing trend of lymphocytes in circulation.

The percentage of monocytes was highest during the 6th month in both male Kangayam calves (8.00 ± 0.26) and female Kangayam calves (6.00 ± 0.33). The neutrophil percent was found to be highest during the 3rdmonth in both male (32.60 ± 0.60 %) and female (30.08 ± 1.35 %) Kangayam calves. The range of values recorded for monocytes (1.8-8.0) and a decreasing trend in the percentage of neutrophils (32-10 %) indicates chances of getting an infection during early life. However, the values were within the physiological range (MSD Veterinary Manual. 2020). Kumar *et al.* (2017) reported a similar range of values of monocyte (3.17-3.83) and neutrophils (32.25-35.50) in Hariana, Sahiwal, and Hardhenu calves of 0 to 1 year of age.

Highly significant ($p \leq 0.01$) difference was observed among age groups for eosinophils percent in both the sexes. A significant increase in the percentage of eosinophils was noticed in both male (5.00 ± 0.26) and female (4.60 ± 0.31) during the 12th month of age compared to other growth phases. However, the values recorded were within the hematological reference range (MSD Veterinary Manual. 2020).

No significant ($p \leq 0.01$) difference was observed among age groups for platelets percent in both the sexes. The values recorded in this study were within the normal physiological range of values (100-800) (MSD Veterinary Manual. 2020).

Highly significant ($p \leq 0.01$) difference was observed among age groups for MCV and MCHC in both the sexes. The MCV, MCHC values are low during the 3rd month, followed by

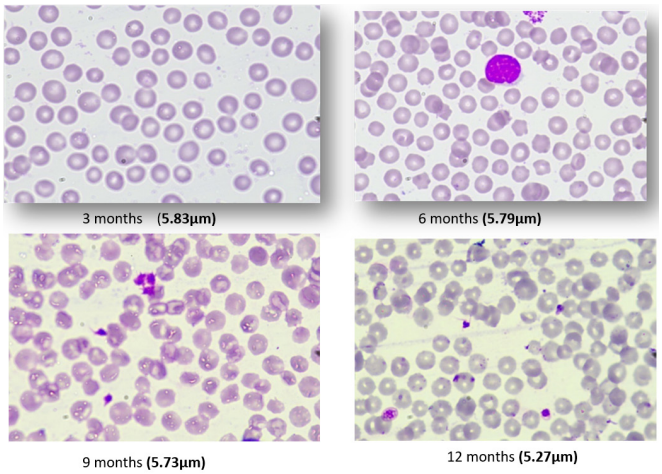

Fig. 1: Average size of RBC (µm) in Kangayam calves from 3 to 12 of age Giemsa stain (100 X)

a gradual increase during the 9th and 12th month and a non-linear increase in MCH during the growth phase of both male and female calves. A similar decreasing trend in MCV and MCHC values was observed by Mohri *et al*. (2007) in Holstein's calves, whereas Kumar *et al*. (2017) reported a higher range of values in Hariana, Sahiwal, and Hardhenu calves (46.88-48.20) of 0-1 year of age. The lower values recorded during the early growth phase might be due to the shift of fetal Hb to adult Hb (Jain. 1986).

A decrease in RBC size (5.8 ± 0.11 to 5.20 ± 0.25 µm) was observed as age advances; however, the difference was non-significant. The size of RBC was slightly bigger in female Kangayam calves than male calves (Fig. 1). The decreasing trend of diameter or length of RBC during the early period of growth might be due to the persistence of red blood cells which formed during embryonic life even after parturition and adaptation of stem cells to new conditions of life after parturition (Adili and Melizi. 2013). However, Dash (2020) reported a highly significant ($p \leq 0.01$) difference in length and breadth of RBCs between age and sex of the animals indicating the profound effect of age and sex on the morphometrics of RBCs in a non-descriptive breed of Odisha. The non-significant reduction in RBC size in the present study might be due to the disappearance of fetal hemoglobin and replacement by adult hemoglobin A (Jain. 1986).

Conclusion

The findings of the present study finding indicates that calves had more RBCs and WBCs and a lower percentage of lymphocytes than the adult reference values. The estimated hematological values will be useful as reference values for indigenous Kangayam calves to the clinicians to diagnose and compare the blood picture during different clinical and some physiological conditions like a different breed, sex, and age. The morphometric study of RBCs will be helpful to diagnose different types of anemia during the growing stage.

Acknowledgment

The authors thank the Dean, Veterinary College and Research Institute, Namakkal, and the Tamil Nadu Veterinary and Animal Sciences University for the successful conduct of Research under the Ph.D. Program

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