



Haematology of *Calotes versicolor* Daudin (1802) in different seasons

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Abstract

The study is aimed to investigate the haemoprofile of common garden lizard in Odisha, India. Ten adult lizards of each sex in different seasons were collected from the coastal area of Rajnagar block of Kendrapara, 754 225, Odisha, located in 20° 20' N to 20° 37' N latitude and 86° 14' E to 87° 01' E longitude. One millilitre of blood was collected from the ventral tail vein and transferred into vials containing ethylenediaminetetraacetic acid (EDTA) and then transported to the laboratory. The haematological parameters such as Hb, PCV, TEC and TLC were determined while erythrocyte indices such as MCV, MCH and MCHC were calculated. The differential leucocyte count (DLC) was also estimated using standard procedures. The haemoglobin concentration is found to be significant at $F_{0.01}$ in both sexes, PCV at $F_{0.001}$ among all males and at $F_{0.05}$ among all females, the TEC at $F_{0.001}$ in all males at $F_{0.01}$ in all females, MCV at $F_{0.001}$ in males and at $F_{0.05}$ in all females and MCH at $F_{0.05}$ in both sexes in three different seasons. The correlation with R^2 values varies in male and female with respect to the parameters analysed. The data obtained could be useful in understanding the seasonal variations on haematological parameters between sexes of *Calotes versicolor*.

Keywords Haemoprofile · Seasons · Significant difference · *Calotes versicolor*

Introduction

Reptiles are the first successful terrestrial air-breathing amniotic vertebrates distinguished by having a dry scaly skin. The haemoprofile of reptiles is necessary for a thorough knowledge about the body physiology which is becoming more crucial due to the economic importance of reptiles, their role as pets and the demand of steps for their conservation. The haematological technique for the assessment of body physiology having a definitive diagnosis is uncomplicated, minimal invasive, productive first-hand technique and economically sustainable.

Common garden lizard belongs to Class-Reptilia, Order-Squamata and Family-Agamidae. It is confined to the Old World, and the majority of genera are found in the Oriental region (Daniel 2002; Das 2010; Das and Das 2017).

Haemoprofile is a very useful and widely used tool that aids in the diagnosis and monitoring of animal health (Sykes and Klaphake 2008; Stacy et al. 2011). According to Behera et al. (2017), different external and internal factors play a role in the haematology of non-mammalian vertebrates. The haematological parameters like haemoglobin, haematocrit value (PCV), mean cell volume, mean cell haemoglobin and mean cell haemoglobin concentration have been examined and reported in some reptiles (Ponsen et al. 2008; Troiano et al. 2008). The haematology in reptiles is highly dependent on seasonal factors, age and sex, and it varies throughout life (Parida et al. 2012, 2013). The quantification of blood is followed as in case of the chelonians (Chansue et al. 2011). The data regarding haematology of reptiles is still challenging in comparison to other non-mammalian vertebrates. Many publications have reported the different aspects of *Calotes versicolor*, but the literature available on haematology of the common garden lizard specifically from the studied geographical area of Odisha is scanty. The comparative scarcity of information regarding season wise haematology of common garden lizards provides less evidence about the family Agamidae. The purpose of this study was to determine the reference intervals of haematological parameters in different seasons between both sexes of normal and healthy common

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garden lizards. The haematological parameters differ in their mean values, and some are also significantly different. The research data will serve as baseline information for these animals.

Materials and methods

Animals

Ten adult lizards of each sex were collected from the coastal area of Rajnagar block of Kendrapara, 754 225, Odisha, India, located in 20° 20' N to 20° 37' N latitude and 86° 14' E to 87° 01' E longitude. The adult lizards were identified based on different morphological characters. Sexual dimorphism was found based on morphometry of body parts. The size of head, limbs, snout to vent length (SVL), and total length including the tail were found to be relatively larger in males, but the abdomen was broader in females. Bright red colouration was more prominent in the throat of adult males in breeding season (Fig. 1). They were categorised into breeding, winter and summer groups. The used superscripts (●) represents breeding, (●●) represents winter and (●●●) is for summer individuals. They were caught during day time (8 a.m. to 4 p.m.) from the gardens and trees. The lizards (Fig. 1) were clinically healthy and in good body condition and then transferred to animal house maintained near the research site. The study was carried out between 2017 and 2020.

Blood collection

The venipuncture site was prepared aseptically prior to blood collection. Blood was collected from the ventral tail vein of lizards by inserting an insulin syringe (BD Ultra – Fine™ Needle 12.7 mm × 30G) at an angle of 45–60° between the scales on ventral midline (Colville and Bassert 2015). Once blood appeared in the needle hub, the needle was held in a steady state, and a gentle negative pressure was applied to the syringe. The blood was kept in an EDTA vial and then transported in icebox to laboratory. The lizards were released to their natural habitat after collection of blood.

Haematological analysis

The whole blood was used for the estimation of haematological parameters. The blood analyses were carried out using the procedure (Saggese 2009; Rizzi et al. 2010; Colville and Bassert 2015; Bassert et al. 2017). The haemoglobin was estimated as oxyhaemoglobin by Sahali's haemometer and expressed in gdl^{-1} . The packed cell volume (PCV) was determined by microhaematocrit method with a spun of microhaematocrit tube at 2500 rpm for

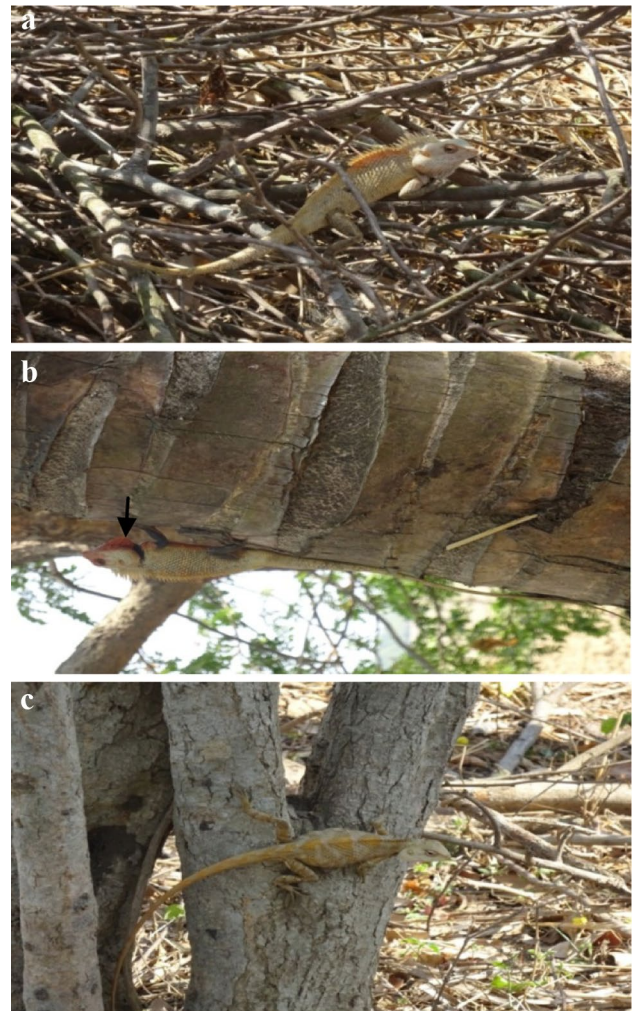


Fig. 1 *Calotes versicolor*. **a** Male. **b** Breeding male. **c** Female

15 min. The quantification of erythrocytes and leucocytes was performed by manual methods using haemocytometer, with Hayem's diluting fluid for erythrocytes and Turk's diluting fluid for leucocytes. Erythrocyte indices like mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated by using standard formulae (Samour 2006; Saggese 2009). The percentage of different leucocytes as well as total platelet count was performed following the method of Campbell et al. 2010.

Statistical analysis

The data presented as mean and standard error (SE) for both sexes and Microsoft office Excel 2007 was used for statistical analysis. The correlation analysis between the parameters was performed. The significant difference ($t_{0.05}$, $t_{0.01}$, $t_{0.001}$) was taken using Student's t -test assuming equal variances, and the F values at ($*F_{0.05}$, $**F_{0.01}$, $***F_{0.001}$) were

Table 1 Haematological parameters of *Calotes versicolor* Daudin, 1802 in different seasons

SL no	Haematological parameters	Unit	Sex	Seasons						F value			
				Breeding		Winter		Summer					
				Range	Mean	SE	Range	Mean	SE		Range	Mean	SE
1	Haemoglobin	gdl ⁻¹	Male	8.39–11.52	10.41 a, c	0.33	5.97–10.4	8.19 c	0.47	6.78–10.8	9.21 a	0.45	6.71 **
			Female	5.65–10.65	8.79 c	0.48	4.53–9.12	6.32 c	0.48	5.76–10.12	8.8	0.47	8.51 **
2	PCV	%	Male	30–39	34.6 e, f	0.97	26–30	24.5 e	1.62	19–35	27.4 f	1.83	11.71 ***
			Female	14–34	26.4 a	2.1	12–30	19.3 a, c	2.1	12–34	27.8 c	2.12	4.84 *
3	TEC	10 ⁶ mm ⁻³	Male	1.11–2.38	1.72 e	0.14	0.75–1.11	0.89	0.03	0.75–1.56	1.004 e	0.07	23.53 ***
			Female	0.75–1.65	1.17 c	0.19	0.71–1.01	0.85 a, c	0.03	0.73–1.21	0.98 a	0.04	6.83 **
4	MCV	fl	Male	151.26–282.26	210.1 d, c	13.87	194.8–333.33	273.85 c	12.63	211.54–346.53	275.17 d	12.93	7.99 **
			Female	172.41–288.28	226.49 b	11.33	160–344.83	226.1 a	19.15	164.38–336.95	284.5 a, b	15.23	4.03 *
5	MCH	pg	Male	44.39–84.84	64.03 e, c	4.64	76.53–101.97	91.59 e	2.88	84.75–192.86	105.49 c	9.93	10.39 ***
			Female	58.9–91.92	76.75 b	3.58	60.4–104.82	74.22 a	4.29	72.87–127.46	90.36 a, b	5.15	3.91 *
6	MCHC	%	Male	23.97–32.94	30.15 a, c	0.85	30.08–41.53	33.81 c	1.06	30.08–39.79	34.11 a	1.01	5.08 *
			Female	29.93–40.35	34.13	1.25	28.72–37.75	33.68	1.16	22.3–47.08	32.93	2.17	0.14 NS
7	TLC	10 ³ mm ⁻³	Male	6.2–11.2	9.15	0.48	6.2–14.5	11.14 a	0.93	5.9–11.2	8832 a	0.57	3.35 *
			Female	5.0–11.0	8.1 c	0.56	6.0–14.5	11.64 c, e	0.79	5.0–10.0	7.88 e	0.55	10.81 ***
8	TPC	10 ³ mm ⁻³	Male	12.6–45.6	30.24	2.82	20.9–42.6	26.38	3.53	19.7–45.8	31.53	2.38	0.82 NS
			Female	18.8–49.5	33.98	3.19	16.5–41.5	26.06	2.51	17.9–91.5	33.02	3.49	2.003 NS

Different lowercase letters indicate significant difference (a, b = $t_{0.05}$, c, d = $t_{0.01}$ and e, f = $t_{0.001}$)

NS non-significant values

* $F_{0.05}$; ** $F_{0.01}$; *** $F_{0.001}$ for each haematological parameter row wise

calculated by analysis of variance (ANOVA) single factor with the help of Paleontological Statistics (PAST) version 2.17 (Natural History Museum, University of Oslo).

Result

In the case of the breeding *C. versicolor*, the haemoglobin concentration, the average haemoglobin content in a single erythrocyte (MCH), and the average percentage of saturation of RBC with haemoglobin (MCHC) between two different sexes were significant ($t_{0.05}$) having t -values 2.75, 2.16, and 2.63 respectively. The packed cell volume in percentage and the total erythrocyte count were significant at $t_{0.01}$ having t -values 3.55 and 3.31. The MCV, TLC and TPC were non-significant. The winter individuals of *C. versicolor* showed a significant difference ($t_{0.05}$) with respect to the haemoglobin concentration, packed cell volume and the total erythrocyte count between male and female individuals ($t=2.77$, 2.12 and 2.28 respectively). In both sexes, the average volume of individual red blood cell was significant at $t_{0.01}$ ($t=3.36$). The TEC, MCHC, TLC and TPC were not significant. The haematological parameters were not significantly different in between male and female individuals of summer adult *C. versicolor*.

The comparative haemoprofile of male and female individuals in three different seasons like breeding, winter and summer is presented in Table 1. The haemoglobin concentration among all males and females in three different seasons was significant ($F_{0.01}$). Likewise, the packed cell volume was significant ($F_{0.001}$) among all males and at $F_{0.05}$ among all females in three different seasons. In three different seasons,

among all males, the total erythrocyte count was significant ($F_{0.001}$), whereas all females are significant at $F_{0.01}$. The average volume of individual red blood cell among all males and females in three different seasons shows significant differences ($F_{0.01}$ and $F_{0.05}$, respectively). The average haemoglobin content in a single red blood cell among all males in three different seasons was significant ($F_{0.001}$), but all females were significant at ($F_{0.05}$). The average percentage of saturation of RBC with haemoglobin showed a significant difference ($F_{0.05}$) among all males in three different seasons. The total leucocyte count among all males in the three different seasons was significant at $F_{0.05}$, and in all female individuals, it was significantly different at $F_{0.001}$.

An assessment on DLC among all males and all females of *C. versicolor* in three different seasons is presented in Table 2. The DLC of *C. versicolor* revealed that the heterophils had the highest percentage of occurrence among all leucocytes followed by lymphocytes. The basophils were found to be of least occurrence among the leucocytes. In breeding male and female, there was no significant difference in DLC. But males showed the highest percentage of heterophils while females showed the highest lymphocytes and monocytes. In this study, the winter females recorded the presence of basophils. The percentage of heterophils was significantly different ($t_{0.05}$) between two sexes having $t=2.21$. The percentage of lymphocytes was highest in females, and eosinophils were found to be highest in males. In summer males and females, the percentage of both heterophils and lymphocytes recorded significant difference ($t_{0.01}$) with t -values 2.94 and 3.11. The monocytes were highest in percentage in comparison to eosinophils between two sexes. The percentage of heterophils among all males in three different seasons showed significant

Table 2 Differential leucocyte count of *Calotes versicolor* Daudin, 1802 in different seasons

SL no	Haematological parameters	Unit	Sex	Seasons									F value
				Breeding			Winter			Summer			
				Range	Mean	SE	Range	Mean	SE	Range	Mean	SE	
1	Heterophil	%	Male	45–60	54.2e	1.5	50–62	57.4a	1.52	60–70	64.3a, e	1.54	11.2***
			Female	35–60	50d	3.17	50–60	53.3c	1.06	52–64	58.5c, d	1.23	4.32*
2	Lymphocytes	%	Male	30–40	36.6	1.2	30–47	36.7	1.8	25–35	30.5	1.26	5.98**
			Female	25–58	39.3	3.6	32–45	40.5	1.2	28–40	36.2	1.33	0.88 NS
3	Eosinophils	%	Male	1–5	2.6	0.49	0–6	3.2	0.64	0–4	1.7	0.39	2.07 NS
			Female	1–7	3.1	0.6	1–5	2.4	0.45	0–4	1.8	0.35	1.8 NS
4	Monocytes	%	Male	4–15	6.6d, c	1.07	1–6	3.1c	0.58	1–6	3.4d	0.65	5.84**
			Female	1–18	8.3a, c	1.65	2–6	3.2c	0.44	2–5	3.5a	0.37	8.0**
5	Basophils	%	Male	0	0	0	0	0	0	0	0	0	0
			Female	0	0	0	0–2	0.4	0.22	0	0	0	3.27 NS

Different lowercase letters indicate significant difference (a, b = $t_{0.05}$, c, d = $t_{0.01}$ and e, f = $t_{0.001}$)

NS non-significant values

* $F_{0.05}$; ** $F_{0.01}$; *** $F_{0.001}$ for each haematological parameters row wise

difference ($F_{0.001}$), but in the case of females, it was significant at $F_{0.05}$. All the males in three different seasons showed a significant difference ($F_{0.01}$) with respect to the percentage of lymphocytes. The percentage of monocytes among all females in different seasons showed significant at $F_{0.01}$.

The comparative picture on correlation between haematological parameters of all male and all female individuals of *C. versicolor* in three different seasons was presented with regression line and R^2 value (Fig. 2 (a–n)). In breeding male and female individuals (Fig. 2 (a*–n*)), the Hb vs PCV,

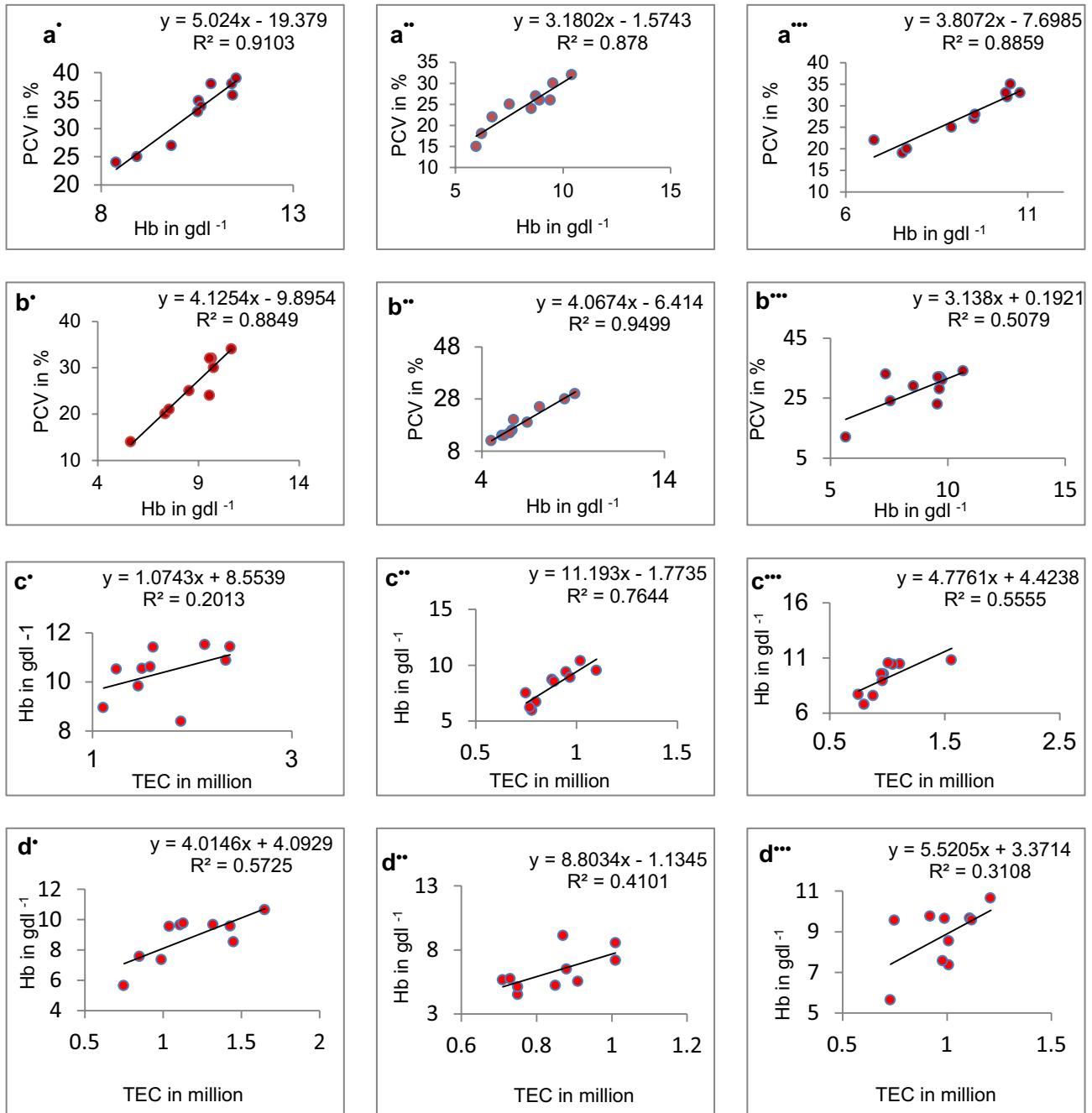


Fig. 2 Comparison on correlation between different haematological parameters (a–n) of *Calotes versicolor* Daudin 1802 in different seasons. (* = Breeding season; ** = Winter season; *** = Summer season). The correlation a*, a** and a*** between Hb vs PCV in male and b*, b** and b*** in female; c*, c** and c*** are TEC vs Hb in male and d*, d** and d*** are in female; e*, e** and e*** are Hb vs MCV in male

and f*, f** and f*** are in female; g*, g** and g*** are MCV vs TEC in male and h*, h** and h*** are in female; i*, ii** and iii*** are MCH vs TEC in male and are j*, j** and j*** in female; k*, k** and k*** are MCV vs MCH in male and l*, l** and l*** are in female; m*, m** and m*** are MCV vs MCHC in male n*, n** and n*** are in female

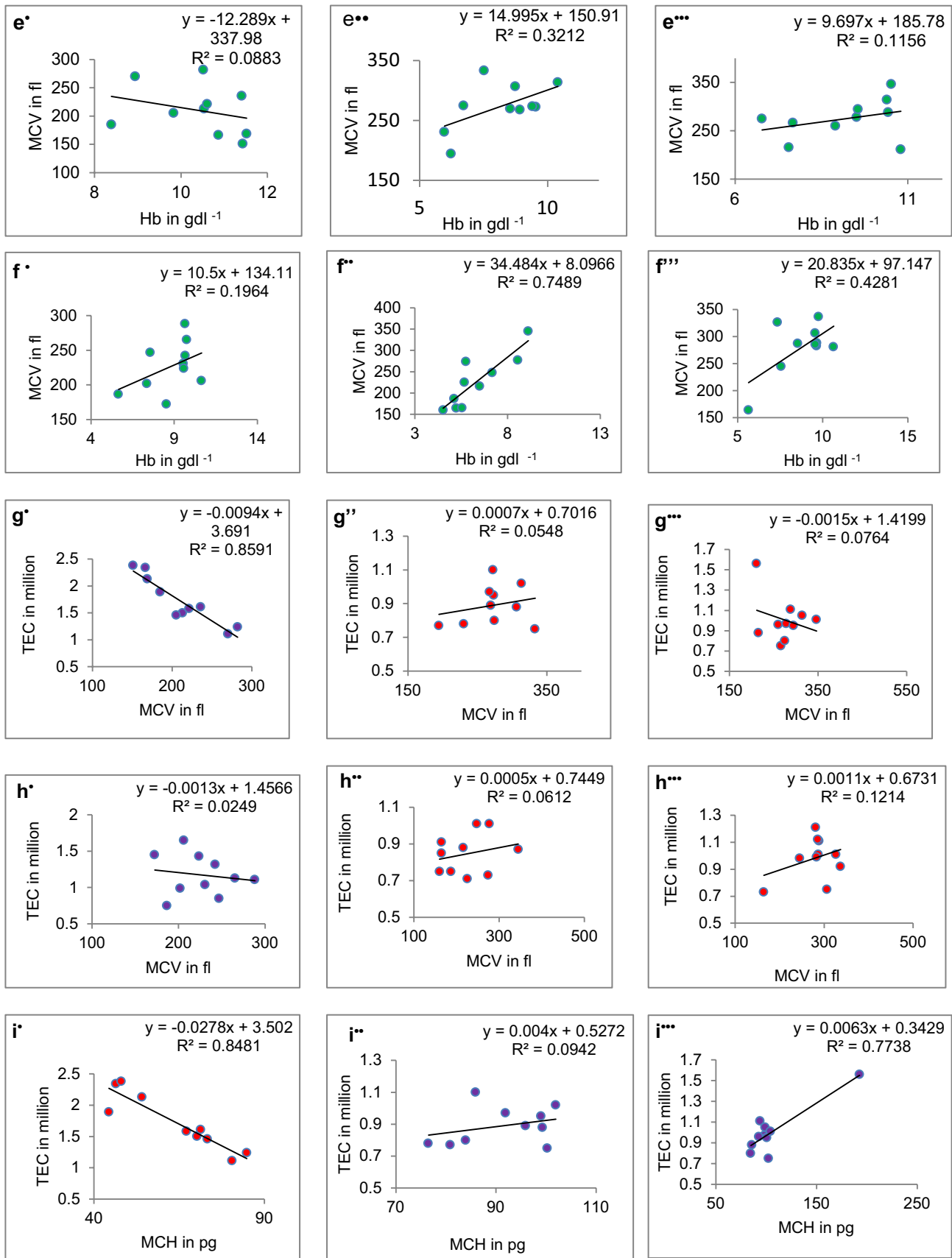


Fig. 2 (continued)

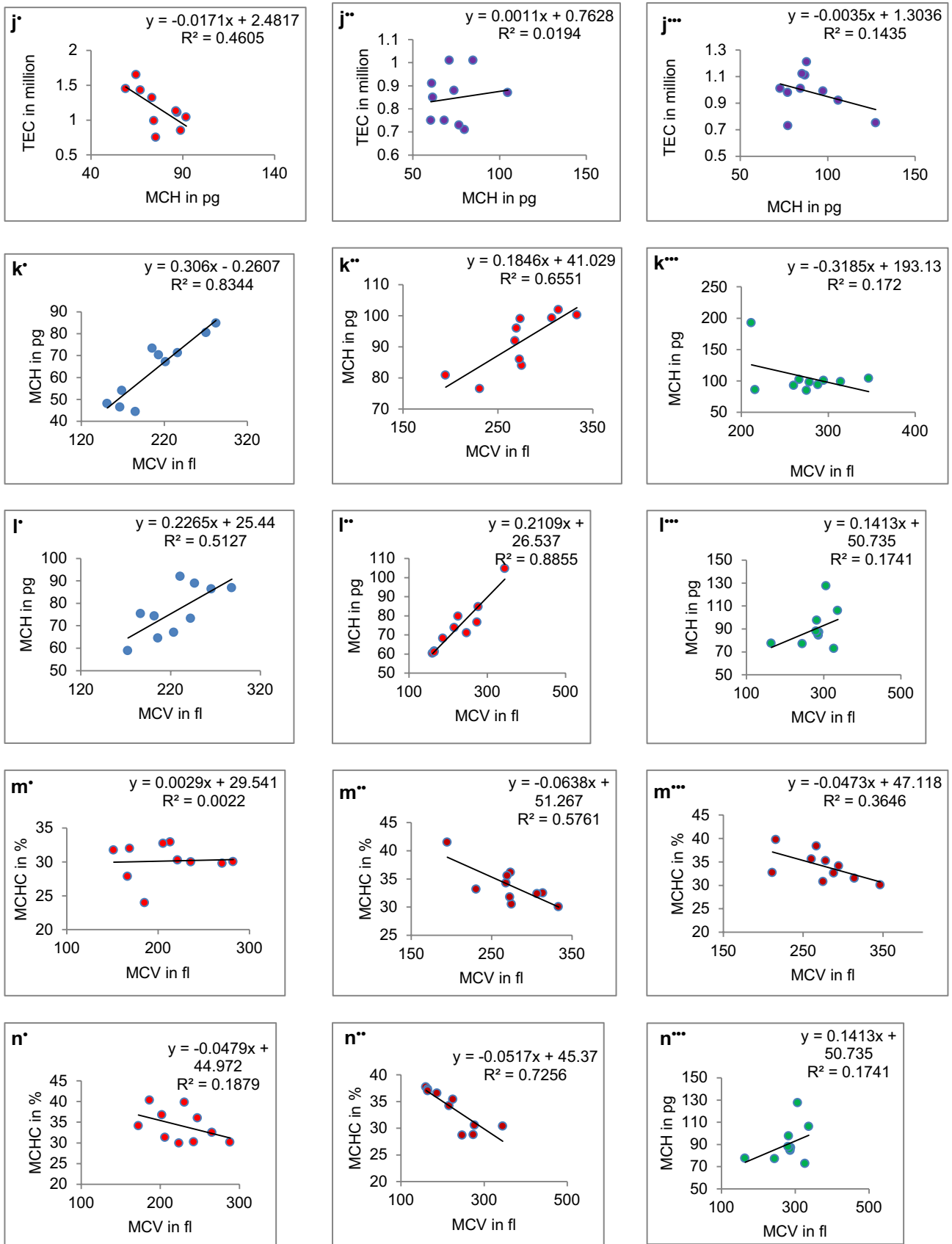


Fig. 2 (continued)

TEC vs Hb and MCV vs MCH were positively correlated, whereas MCV vs TEC and MCH vs TEC were negatively correlated, but Hb vs MCV showed negative correlation in male and positive in female and MCV vs MCHC was positively correlated in males and negatively in females. In the case of winter individuals (Fig. 2 (a^{••}–n^{••})), the correlation between parameters, such as Hb vs PCV, TEC vs Hb, Hb vs MCV, MCV vs TEC, MCH vs TEC and MCV vs MCV, was found to be positive. In both sexes, the MCV and MCHC were negatively correlated.

In summer individuals (Fig. 2 (a^{•••}–n^{•••})), the parameters such as Hb vs PCV, TEC vs Hb and Hb vs MCV were positively correlated. The MCV and TEC were negatively correlated in males and positively in females. The correlation between MCH and TEC was positive in males and negative in females. The male individuals exhibited a negative correlation with MCV vs MCH, whereas in the females, the correlation was positive. The MCV and MCHC were negatively correlated in both sexes.

Discussion

The haemoprofile of *Calotes versicolor* differs in male and female and also varies with respect to different seasons. The difference may be due to some seasonal factors in combination with age and sex of the individuals (Rossini et al. 2011; Lisicic et al. 2013; Parida et al. 2013). The mean value of some haemo parameters differs between sexes and also varies in different seasons. The Hb and TEC of *C. versicolor* are found to be higher in comparison to other agamids (Parida et al. 2012), but the TLC is lower in *C. versicolor*. The haemoprofiles as studied by Lisicic et al. (2013) varies in accordance to sex, and our data also matches with the same concept of variation. In this study, it is noted that haemogram varies with sex, and it is similar to some studied sand lizards (Ponsen et al. 2008) and are higher in both sexes of *C. versicolor* in comparison to other gecko species (Olayemi 2011; Nayak and Mohanty 2020). But in our study, no basophils were observed in male lizards. According to Bailey et al. (2011), *Heloderma suspectum* shows higher PCV and lower in TEC, Hb, percentage of heterophils and lymphocytes. The data regarding concentration of Hb, TEC, TLC, percentage of heterophils and lymphocytes show closeness with the finding of some Agamidae lizards with some significant differences in parameters (Pal et al. 2008). The TEC, TLC, MCV, MCH and MCHC in both male and female *Naja naja* are higher (Parida et al. 2014) in comparison to *C. versicolor* but the haemoglobin is found to be higher in all except winter females, whereas the PCV is lower in both sexes of winter male *C. versicolor*. The heterophil count and the lymphocyte count are higher, and eosinophil and monocyte

percentage is found to be lower in both sexes in this study in comparison to *Psammophilus blanfordans* (Parida et al. 2012). The monocyte and eosinophil percentage is highest, and heterophil and lymphocyte percentage is lowest in some other agamid lizard species (Gul and Tosunoglu 2011). According to Smyth et al. (2017), the heterophils and lymphocytes are the highest occurred leucocytes followed by monocytes and eosinophils. The differential leucocyte count of *C. versicolor* shows some similarity but the percentage of heterophils is higher, and lymphocyte is lower in this study in comparison to other lacertid lizards (Sachi et al. 2011).

The correlation between haematological parameters also falls within the range of geckonid lizards (Nayak and Mohanty 2018, 2020) and of some non-mammalian vertebrates (Acharya and Mohanty 2018). The MCV and MCHC are found to be the highest in both sexes in comparison to *Heloderma suspectum* (Bailey et al. 2011).

In common garden lizard, the PCV and TEC are directly proportional to concentration of haemoglobin. The breeding male individuals show an indication of poor health status like anaemia. The results of our study corroborate these evidences.

Conclusion

The present study re-established the data on haemoprofile of *Calotes versicolor* between sexes in different seasons. We were also able to demonstrate the seasonal variation in the haematology of common garden lizard. This present investigation provides a baseline reference value for the haematological parameters of agamids with reference to different seasons. Further blood profiling may also be helpful in detecting the health issues. Ongoing and further monitoring studies may assess the information to check the physiology of agamids in different seasons. The data reported in this study represent an important step toward normal range on haematology of common garden lizard can be compared in other agamids.

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Declarations

Ethics approval The investigation followed all the guidelines and care of animals.

Conflict of interest The authors declare no competing interests.

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