Comparative Clinical Pathology (2020) 29:275–281
https://doi.org/10.1007/s00580-019-03051-5

ORIGINAL ARTICLE

Haemoprofile of yellow-bellied house gecko, *Hemidactylus flaviviridis* Ruppell, 1835

Sarbeswar Nayak[1] · Prafulla Kumar Mohanty[2]

Received: 4 September 2018 / Accepted: 16 August 2019 / Published online: 2 September 2019

Abstract

The study is carried out to investigate the haemogram of yellow-bellied house gecko in Odisha, India. Fifteen adult lizards of each sex 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 in icebox to laboratory. The morphology of blood cells along with haematological parameters like haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), total leucocyte count (TLC), total platelet count (TPC), and differential leucocyte count (DLC) was calculated using standard procedures. Statistical analyses like correlation and *t* test were done. The study revealed that the TLC shows significant difference ($t_{0.01}$) while the percentage of eosinophils is found to be significant at $t_{0.05}$ between both sexes of lizard. The mean values of all other parameters are also showing difference between males and females of *Hemidactylus flaviviridis* Ruppell, 1835. The correlation with R^2 values varies in males and females with respect to the parameters analysed. Some parameters are positively correlated with each other, and others are found to be negatively correlated. The data obtained could be useful in knowing the difference of haematological parameters between sexes of *Hemidactylus flaviviridis*.

Keywords *Hemidactylus flaviviridis* · Sexes · Haemogram · Correlation · Significant difference

Introduction

The worth of paying attention to haematological study for analysing, evaluating, examining, assessing, and controlling the health status of animals is a prelude blueprint. The haematological technique for assessment of body physiology has a definitive diagnosis is uncomplicated, minimal invasive, productive first-hand technique, and economically sustainable.

Yellow-bellied house gecko is belonging to the class Reptilia, order Squamata, and family Gekkonidae, found in warm climatic conditions throughout the world (Das 2010; Das and Das 2017). Haemocytological parameters are very useful and widely used tools that aid in the diagnosis and monitoring of animal health (Sykes and Klaphake 2008; Cheville 2009; Rovira 2010; Stacy et al. 2011). The integration of different haematological parameters diagnoses the physiological conditions and clinical evaluation in reptiles (Vasaruchapong et al. 2013). The different external and internal factors play a role in the haematology of nonmammalian vertebrates (Behera et al. 2017). The 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 age as well as sex and it varies throughout life (Parida et al. 2012, 2013). The blood cells are identified and different examined parameters are taken into account (Reagan et al. 2008; Boudreaux 2010; Jensen and Kjelgaard-Hansen 2010; Chansue et al. 2011). The data regarding haematology of reptiles is still challenging in comparison with other nonmammalian vertebrates. The literature available on

✉ Sarbeswar Nayak
nayaksarbeswar89@gmail.com

Prafulla Kumar Mohanty
prafulla.mohanty3@gmail.com

[1] Department of Zoology, Vikram Deb Autonomous College, Jeypore, Koraput 764 001, India

[2] Department of Zoology, Utkal University, Vani Vihar, Bhubaneswar 751 004, India

haematology of this lizard specifically from the studied geographical region of Odisha is very less. The comparative scarcity of information regarding the haematology of geckos provides a less evidence about the family Gekkonidae. The purpose of this study was to determine the reference intervals of haematological parameters between different sexes of normal and healthy geckos. The morphology of blood cells is almost similar in both sexes, but the haematological parameters differ in their mean values and some are also significantly different. The findings will serve as baseline data for future health assessment of lizards.

Materials and methods

Animals

Fifteen adult lizards of each sex 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. They were caught at evening near by the light sources on walls and boundaries and transferred to animal house. The lizards were clinically healthy and in a good body condition. The investigation of haematological profiles on *Hemidactylus flaviviridis* Ruppell, 1835 was carried out from 2014 to 2017.

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 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. Whole blood smear was obtained by push-slide technique, air-dried, fixed with methanol, and stained with Giemsa as protocol cited by Lillie (1977).

Haematological analysis

The blood analysis was 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 Sahli's haemometer and expressed in g%; packed cell volume (PCV) was determined by the microhaematocrit method with a spun of microhaematocrit tube at 2500 rpm for 15 min. The quantification of RBCs and WBCs was performed by manual methods using haemocytometer, with Hayem's diluting fluid for RBCs and Turk's diluting fluid for WBCs. Erythrocyte indices like MCV, MCH, and MCHC were calculated using standard formulae (Saggese 2009). The percentage of different leucocytes as well as total platelet count was determined (Campbell et al. 2010; Thrall et al. 2012).

Photomicrography

The morphological results of blood cells of the lizards are presented in form of photomicrographs. Well-stained ideal blood smear is chosen for this purpose. The tail ends of the smears are examined under high-power objectives and photographed by a photographic microscope (Hund, Wetzlar, Germany) at × 400 magnification.

Statistical analysis

The data is 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, and the significant difference ($t_{0.05}$, $t_{0.01}$) was taken using Student's *t* test (assuming equal variances) with the help of Paleontological Statistics (PAST) version 2.17 (Natural History Museum, University of Oslo).

Result

The morphology of different blood cells of *H. flaviviridis* is depicted (Fig. 1a–i). The erythrocytes (Fig. 1a) are oval cells and their nuclei are also oval and centrally located. The cytoplasm of mature erythrocytes appeared yellowish and homogeneous. The erythrocytes of abnormal shape observed in these lizards are dacryocytes (Fig. 1b) which are tear drop or spear shaped in appearance. Other types of abnormal erythrocytes are acanthocytes (Fig. 1c), with club-shaped spicules of different lengths projecting from their surface at irregular intervals. These are irregular creneted cells. The elliptical erythrocytes are also found with a distinct clear elliptical centrally located nucleus (Fig. 1d).

The megakaryocytic fragments, the thrombocytes (Fig. 1e) are small and irregular in shape and arranged in a clump. The heterophils (Fig. 1d) are observed with four to five lobes in the nucleus in both the sexes. The monocytes (Fig. 1f) have a distinct bean-seed-shaped nucleus. The lymphocytes, both large and small, are rounded or spherical in shape with a high nuclear cytoplasmic ratio. A narrow rim of light-violet cytoplasm is observed (Fig. 1g, h). The eosinophils (Fig. 1i) having a spectacle-shaped nucleus are circular in appearance.

The haematological parameters are analysed in case of adult healthy lizards (Table 1). Assuming a confidence level less than 1 in 20 chance 100 chance of being wrong,

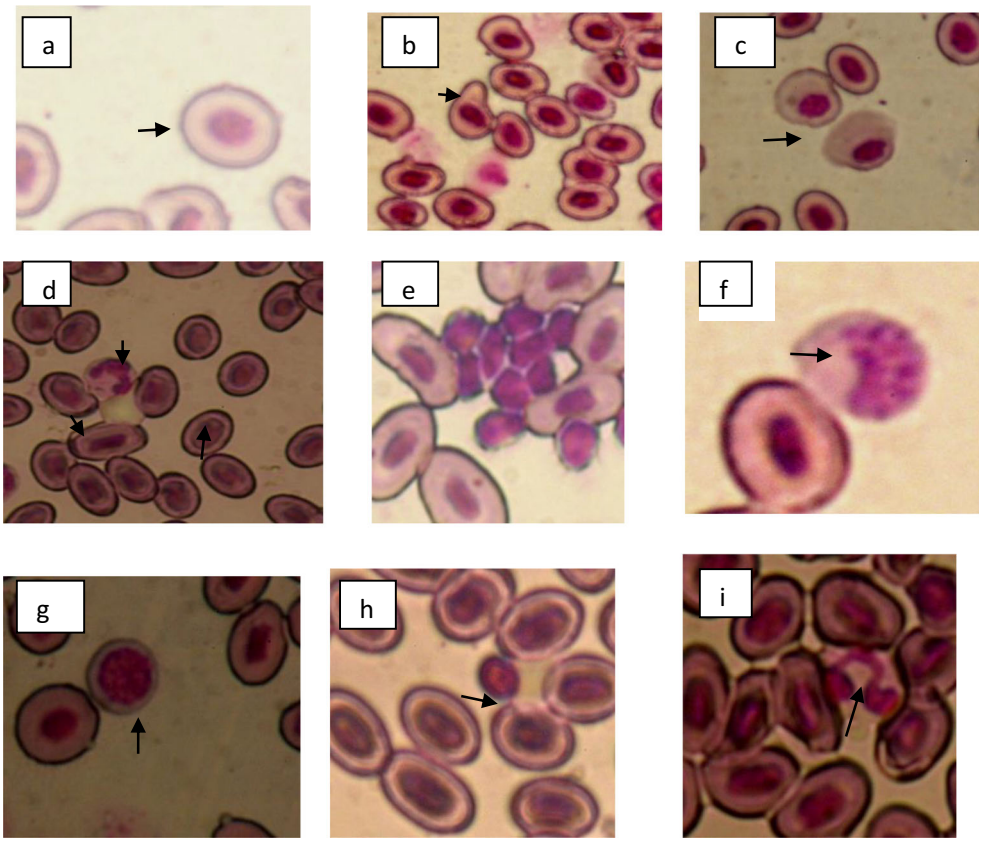

Fig. 1 Morphology of blood cells (**a** rounded erythrocyte, **b** dacryocytes, **c** acanthocytes, **d** heterophil, elliptical erythrocyte, **e** thrombocytes, **f** monocyte, **g** large lymphocyte, **h** small lymphocyte, and **i** eosinophil)

significant differences in haematological parameters are found in two parameters throughout the investigation. The total leucocyte count in thousands per cubic millimetre of blood is found to be the highest in males than in females ($t_{0.01}$). The percentage of eosinophils shows a significant difference ($t_{0.05}$) between two sexes. The mean values of haemoglobin, PCV, MCV, MCH, MCHC, TPC, and percentage of heterophils are found to be the highest in females while the TEC and the percentage of lymphocytes and monocytes are found to be the highest in male individuals. The basophils are not observed in this investigation (Table 1).

Table 1 Haematological parameters of *H. flaviviridis* Ruppell, 1835

SL no.	Parameters	Unit	Male			Female			*t* value
			Range	Mean	SE	Range	Mean	SE	
1	Haemoglobin	g%	6.43–10.76	7.97	0.32	6.34–9.99	8.03	0.33	0.13
2	PCV	%	17.89–30.76	22.96	1.02	15.67–30.89	24.25	1.18	0.82
3	TEC	10^6 mm^{-3}	1.34–2.67	1.9	0.1	1.01–2.98	1.83	0.18	0.43
4	MCV	fl	90.35–183.13	124.69	7.11	91.006–228.69	145.71	11.73	1.53
5	MCH	pg	31.89–58.73	43.19	2.12	29.76–65.95	47.82	3.2	1.23
6	MCHC	%	30.48–40.13	34.89	0.69	25.77–76	36.13	3.1	0.39
7	TLC	10^3 mm^{-3}	8.96–14.98	12.71	4.63	7.86–13.45	10.86	4.39	2.98**
8	TPC	10^3 mm^{-3}	15.4–35.8	28.43	2.24	12.87–46.78	30.26	2.74	0.52
9	Heterophils	%	40–62	55.27	1.73	30–76	61.2	3.06	1.69
10	Lymphocytes	%	25–45	35.6	1.42	14–62	33	3.18	0.75
11	Eosinophils	%	4–12	5.87	0.56	1–8	4.06	0.49	2.51*
12	Monocytes	%	0–9	3	0.62	0–4	1.8	0.27	1.77

The significant difference *$t_{0.05}$ and **$t_{0.01}$ for each haematological parameters; *SE* standard error of mean

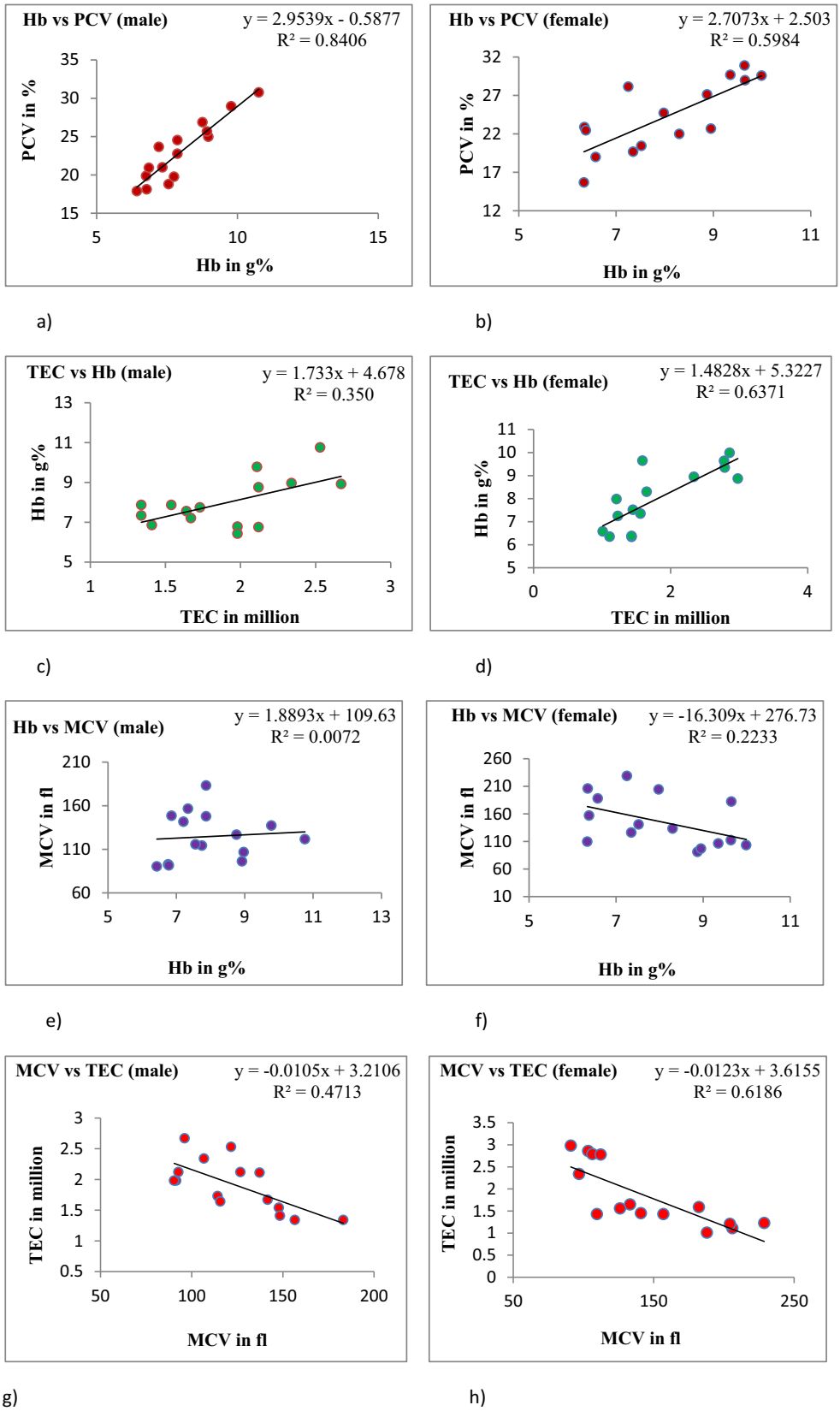

Fig. 2 Correlation between different haematological parameters (**a**–**n**) of *Hemidactylus flaviviridis* Ruppell, 1835

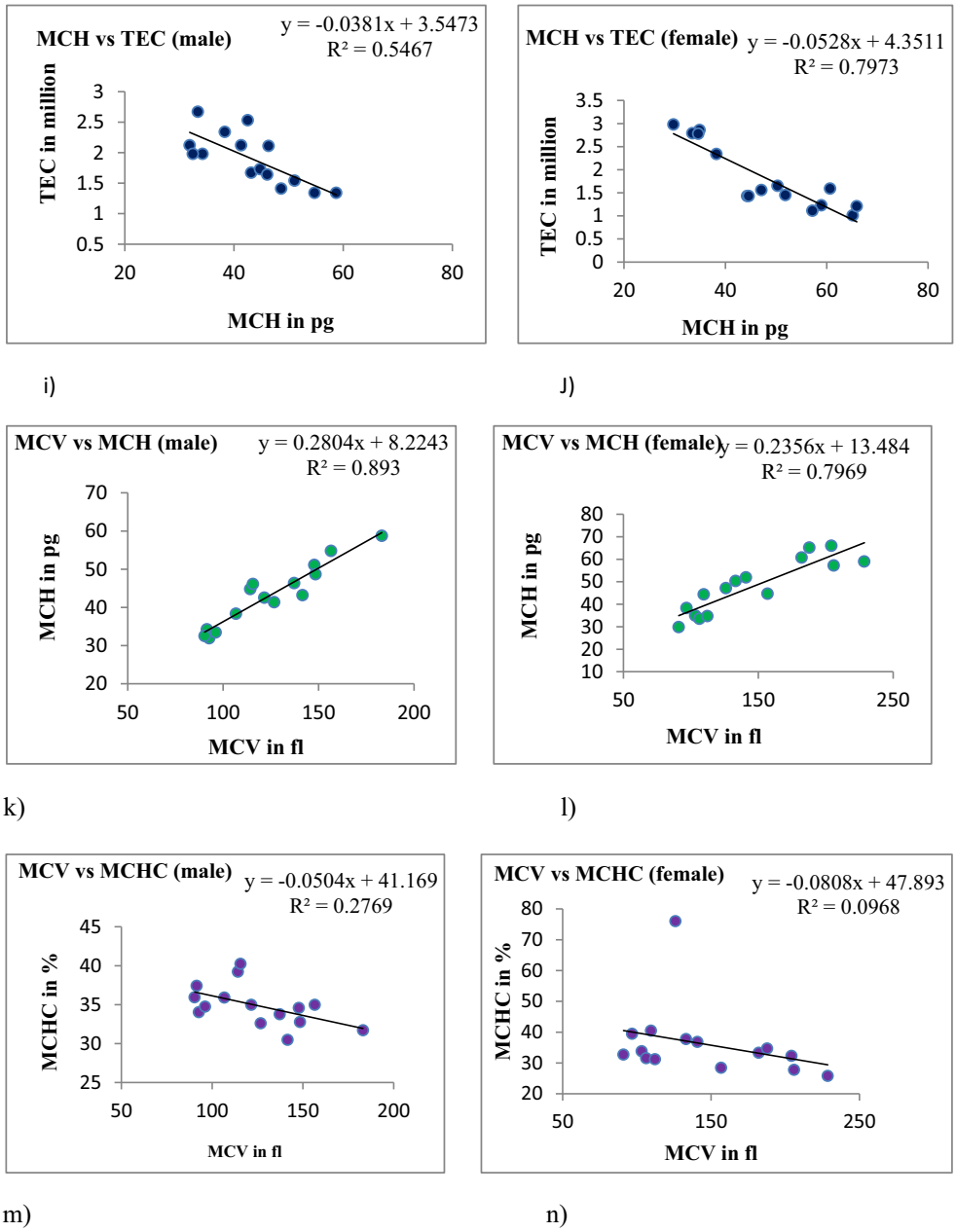

Fig. 2 (continued)

The correlation between various haematological parameters with the linear regression line and R^2 value among male and female individuals of *H. flaviviridis* is depicted (Fig. 2a–n). There is a positive correlation between Hb vs PCV (Fig. 2a, b), Hb vs TEC (Fig. 2c, d), and MCV vs MCH (Fig. 2k, l) in both males and females, but Hb vs MCV (Fig. 2e) is positively correlated in males and negatively correlated in females (Fig. 2f). A negative correlation was found in both sexes with respect to MCV vs TEC (Fig. 2g, h), MCH vs TEC (Fig. 2i, j), and MCV vs MCHC (Fig. 2m, n).

Discussion

The haematological parameters differ in male and female reptiles. The difference may be due to some seasonal factors in combination with age and sex of the individuals (Rossini et al. 2011; Parida et al. 2013). The TEC, TLC, TPC, and percentage of heterophils show the lower value, and g% of haemoglobin and percentage of heterophils show the higher value in both sexes; monocytes and eosinophils are found to be the highest in males, and PCV is higher in females of the

studied gecko in comparison with *Hemidactylus frenatus* (Olayemi 2011). Our findings of TEC and TLC show closeness with the finding of some Agamidae lizards (Pal et al. 2008). In this study, it is noted that haemogram varies with sex. The result obtained in this study regarding the haematological parameters falls within the range as studied in sand lizards (Ponsen et al. 2008). The TEC, Hb, and percentage of heterophils and lymphocytes in both males and females are higher in comparison with *Heloderma suspectum* (Bailey et al. 2011). The PCV, MCV, MCH, and MCHC in both males and females of *Naja naja* are higher (Parida et al. 2014) in comparison with *Hemidactylus flaviviridis*, but the haemoglobin is found to be higher in males of *H. flaviviridis.* The heterophil count, the lymphocyte count, and the g% of haemoglobin is higher in both males and females in this study in comparison with *Psammophilus blanfordans*, and eosinophil and monocyte percentages were found to be lower in both sexes (Parida et al. 2012). The monocyte, eosinophil, and lymphocyte percentages are highest, and the heterophil percentage is lowest in *Trapelus lessonae* (Gul and Tosunoglu 2011). Generally the heterophils and lymphocytes are the highest occurred leucocytes followed by eosinophils and monocytes (Smyth et al. 2017).

In yellow-bellied house gecko, the PCV shows a positive correlation with increase in haemoglobin concentration in both males and females; this indicates that TEC is also directly proportional to the concentration of haemoglobin. With increase in TEC, the PCV, and concentration of haemoglobin, both increased in different sexes and are correlated positively. With increase in MCV, the MCH is increasing in males and females. In case of males, the MCV is increasing with increase in concentration of haemoglobin where as in females, it is negatively correlated. This is an indication of poor health status like anaemia in case of female lizards. TEC shows a negative correlation with MCV and MCH in case of both males and females and the MCV is also negatively correlated with MCHC in both sexes (Nayak and Mohanty 2018), and the correlation also falls within the range in case of some non-mammalian vertebrates (Acharya and Mohanty 2018). The MCV is found to be the lowest and MCHC is the highest in both sexes in comparison with *Heloderma suspectum* (Bailey et al. 2011), whereas MCV, MCH, and MCHC show a higher value in comparison with *Pelusios sinuatus* (Omonona et al. 2011). The result of our study corroborates with these evidences.

Conclusion

The present systematic examination re-established the fact on haemogram of *Hemidactylus flaviviridis* between different sexes. The present investigation provides a baseline reference value for the haematological parameters of geckos. Further blood profiling is helpful in detecting health issues, parasitism, and subclinical metabolic conditions. Ongoing and future monitoring studies may asses the information to check the physiology of geckos.

Acknowledgements The authors express their gratefulness to Postgraduate Department of Zoology, Vikram Deb Autonomous College, Jeypore, Koraput, 764 001, for support and Postgraduate Department of Zoology, Utkal University, Vani Vihar, Bhubaneswar, 751 004, for providing laboratory facilities.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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

References

Acharya G, Mohanty PK (2018) Effect of sex on haemocytobiochemical profiling of silver tigerfish *Datnioides polota* Hamilton, 1822. Comp Clin Pathol 27(5):1335–1342

Bailey KC, Smith SA, Zimmerman K, Lane R, Raskin RE, DeNardo D (2011) Hematology, leukocyte cytochemical analysis, plasma biochemistry, and plasma electrophoresis of wild-caught and captive-bred Gila monsters (*Heloderma suspectum*). Vet Clin Pathol 40(3): 316–323

Bassert JM, Samples OM, Beal AD (2017) McCurnin's clinical textbook for veterinary technicians, 9th edn. Elsevier- Health Science Division, Saunders, Philadelphia, pp 1–456

Behera Y, Nayak S, Mohanty PK (2017) Age and season wise haematological profile of little egret (*Egretta garzetta*) of Chilika wetland. India. Asian J Anim Sci 11(4):158–164

Boudreaux MA (2010) Platelet structure. Chapter 75. In: Weiss DJ, Wardrop KJ (eds) Schalm's veterinary hematology. Blackwell Publishing Limited, Iowa, pp 561–568

Campbell TW, Smith S, Zimmerman L (2010) Haematology of waterfowls and raptors. In: Weiss DJ, Wardrpo KJ (eds) 6th ednSchalm's Vet Hematol. Wiley-Blackwell Publication, New Jersy, pp 977–986

Chansue N, Sailasuta A, Tangtrongpiros J, Wangnaitha S, Assawawongkasem N (2011) Hematology and clinical chemistry of adult yellow-headed temple turtles (*Hieremys annandalii*). Vet Clin Pathol 40:174–184

Cheville NF (2009) Ultrastructural pathology: the comparative cellular basis of disease, 2nd edn. Wiley-Blackwell Publication, Ames, Iowa, pp 1–1000

Colville T, Bassert JM (2015) Laboratory manual for clinical anatomy and physiology for veterinary technicians, 3rd edn. Mosby, St. Louis, Missouri pp 1–656

Das I (2010) A field guide to the reptiles of Southeast Asia. New Holland Publishers (UK), Ltd, London, pp 1–56

Das I, Das A (2017) A naturalist's guide to the reptiles of India, Bangladesh, Bhutan, Nepal, Pakistan and Sri Lanka. John Beaufoy Publishing Limited, Woodstock Road, Oxford, England, pp 1–176

Gul C, Tosunoglu M (2011) Haematological reference intervals of four agamid lizard species from Turkey (Squamata: Sauria: Agamidae). Herpetozoa 24(1/2):51–59

Jensen AL, Kjelgaard-Hansen M (2010) Diagnostic test validation. Chapter 130. In: Weiss DJ, Wardro KJ (eds) Schalm's veterinary hematology. Blackwell Publishing Limited, Iowa, pp 1027–1033

Lillie RD (1977) Methods for testing biological stains. In: Conn's HJ biological stains, 9th edn. The Williams and Wilkins Company, Baltimore, USA, pp 606–607

Nayak S, Mohanty PK (2018) Haematological analysis of leschenault's leaf toad gecko, Hemidactylus leschenaultii Dumeril and Bibron, 1836. Indian J Biol 5(1):53–59

Olayemi OA (2011) Hematological parameters of house gecko (*Hemidactylus frenatus*) in Ibadan Metropolis, Nigeria. Medwell J Vet Res 4(3):77–80

Omonona A, Olukole SG, Fushe FA (2011) Haematology and serum biochemical parameters in free ranging African side neck turtle (*Pelusios sinuatus*) in Ibadan, Nigeria. Acta Herpetol 6(2):267–274

Pal A, Parida SP, Swain MM (2008) Hematological and plasma biochemistry in fan-throated lizard, *Sitana ponticeriana* (Sauria: Agamidae). Rus J Herp Rus 15(2):110–116

Parida SP, Dutta SK, Pal A (2012) Hematological and plasma biochemistry in *Psammophilus blanfordanus* (Sauria: Agamidae). Comp Clin Pathol 21:1387–1394

Parida SP, Dutta SK, Pal A (2013) Hematology and plasma chemistry of wild keeled Indian Mabuya, *Eutropis carinata* (Schneider 1801). Comp Clin Pathol 22:869–873

Parida SP, Dutta SK, Pal A (2014) Hematology and plasma biochemistry of wild-caught Indian cobra *Naja naja* (Linnaeus, 1758). J Venomous Anim Toxins Incl Trop Dis 20:14

Ponsen S, Talabmook C, Narkkong N, Aengwanich W (2008) Blood cell characteristics and some hematological values of sand lizards (*Leiolepis belliana rubritaeniata* Mertens 1961) of Northeastern Thailand. Int J Zool Res 4(2):119–123

Reagan WJ, Rovira ARI, DeNicola DB (2008) Reptilian hematology. Chapter 12, in: veterinary hematology: atlas of common domestic and non-domestic species. Wiley-Blackwell, Iowa, pp 85–95

Rizzi TE, Meinkoth JH, Clinkenbeard KD (2010) Normal hematology of the dog, chapter 104. In: Weiss DJ, Wardrop KJ (eds) Schalm's veterinary hematology. Blackwell Publishing Incorporated, Iowa, p 804

Rossini M, Garcia G, Rojas J, Zerpa H (2011) Hematologic and serum biochemical reference values for the wild spectacled Caiman, *Caiman crocodilus crocodilus*, from the Venezuelan plains. Vet Clin Pathol 40:374–379

Rovira ARI (2010) Hematology of reptiles. Chapter 127. In: Weiss DJ, Wardrop KJ (eds) Schalm's veterinary hematology. Blackwell Publishing Limited, Iowa, pp 1004–1012

Saggese M (2009) Clinical approach to the anaemic reptile. J Exotic Pet Med 18:98–111

Smyth AK, Smee E, Godfrey SS, Crowther M, Phalen D (2017) The use of body condition and haematology to detect widespread threatening processes in sleepy lizards (*Tiliqua rugosa*) in two agricultural environments. R Soc Open Sci 1:1–13

Stacy NI, Alleman AR, Sayler KA (2011) Diagnostic hematology of reptiles. Clin Lab Med 31:87–108

Sykes JM, Klaphake E (2008) Reptile hematology. Vet Clin North Am Exot Anim Pract 11:481–500

Thrall MA, Weiser G, Allison R, Campbell TW (2012) Veterinary hematology and clinical chemistry. John Wiley and Sons, New Jersey, pp 237–273

Troiano JC, Gould EG, Gould I (2008) Hematological reference intervals in Argentina lizard *Tupinambis merianae* (Sauria-Teiidae). Comp Clin Pathol 17:93–97

Vasaruchapong T, Disarapong P, Chulasugandha P, Khow O, Chanhome L, Chiobamroongkiat M, Chaiyabutr N, Sitprija V (2014) Comparative studies on hematological and plasma biochemical parameters in different types of venomous snakes in Thailand. Comp Clin Pathol 23(4):955–959. https://doi.org/10.1007/s00580-013-1721-9