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Comparative Seasonal Haematology of Two Bat Species, *Scotophilus heathii* and *Pipistrellus pipistrellus*, in a Subtropical Area of Pakistan

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A B S T R A C T

Various haematological parameters of two bat species belonging to the family Vespertilionidae (*Scotophilus healthii* and *Pipistrellus pipistrellus*) were examined on the seasonal basis. Blood samples were taken from 47 bats (23 *S. heathii* and 24 *P. pipistrellus*) captured from Potohar region. Results showed that *P. pipistrellus* exhibited significantly (P<0.05) higher mean counts of red blood cells (RBCs), white blood cells (WBCs), platelets, haemoglobin (Hb), and packed cell volume (PCV) as compared to *S. heathii.* However, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) levels were significantly (P<0.05) higher in *S. heathii* as compared to *P. pipistrellus.* Seasonal comparisons in both the species depicted lowest levels of RBCs, WBCs, MCV, MCH and PCV in spring when bats are least active just after hibernation. Highest mean estimates of RBCs, Hb, and PCV were observed in summer (bats are most active) in male and female bats belonging to both species. However, WBCs levels were at peak during autumn when bats are preparing to go into hibernation.

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Authors' Contribution
MI conceived the project and analyzed the data. AS helped in field work and blood sampling. NR analyzed the samples and wrote the article. MI and MSN helped in preparation of manuscript. MSN supervised the work.

Key words
Seasonal variations, Haematological parameters, Vespertilionid bat, ***S. heathii.***

INTRODUCTION

Microchiropterans particularly vespertilionid bats feed on insects and may play an important role in the agro-ecosystems of Pakistan. In spite of their usefulness, bats are symbol of fear and disgust in Pakistan and among the least studied mammals in Pakistan (Robert, 1997; Walker and Molur, 2003; Mahmood-ul-Hassan and Nameer, 2006). A few studies have been conducted on the ecology and distribution of bats in Pakistan (Mirza, 1967; Eates, 1968; Murray, 1884; Nadeem *et al*., 2013). A greater understanding of the biology of bats may be essential for the development of future management or conservation strategies. In this context, seasonal hematological studies are very important which are required for the differential diagnosis of normal and pathological conditions (Gulland and Hawkey, 1990; Campbell, 1992). Blood profile also provides assistance in the studies based on nutritional status of animal populations, which are conducted for the evaluation of their physiological conditions (Dawson and Bortolotti, 1997; Heard and Whittier, 1997). The blood profile is affected by a number of factors which may be external such as the season, time of the day, food availability and quality as well as by age, gender, reproductive state and the changes in the concentration of different metabolites inside the body of an animal (Wolkers *et al.*, 1994; Minemastue *et al*., 1995).

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Keeping this in view we hypothesized that blood profile tends to change with the change of season, gender and species in microchiroptera. Furthermore, the time of activity may have an association with the levels of haematological parameters in the hibernating mammals. The present study was designed to investigate seasonal variations in haematological parameters of two species of vespertilionid bats; *Scotophilus heathii* and *Pipistrellus pipistrellus,* widely distributed in Pakistan . These species reproduce in spring and early summer (March to May) after arising from hibernation (January to February), and hibernate in winter (December to January).

MATERIALS AND METHODS

Study area

The present study was conducted in the Potohar plateau (32.5°N to 34.0°N Latitude and 72°E to 74°E Longitude), a subtropical arid region with a 350-500 mm/ annum rainfall, is forming the northern part of the province Punjab, Pakistan. The average temperature varies from 7.9ºC in winter to 30.6ºC in summer.

Sample collection

A total of 47 microbats belonging to two species, *i.e. Scotophilus heathii* (n=23; 12 male and 11 female) and *Pipistrellus pipistrellus* (n=24; 12 male and 12 female) were subjected to the present study. Bats were collected throughout the year from different areas in the Potohar region by using mist-net. As both the bat species are hibernating, specimens were not found during winter season (December to February) (Table I). After capturing, bats were immediately brought to the Wildlife Laboratory, Department of Zoology, Pir Mehr Ali Shah, Arid Agriculture University Rawalpindi and identified by using standard key (Bates and Harrison, 1997). The blood samples of the captured adult bats were taken in heparinized syringes through median venipuncture, and poured in the heparinized vacutainers.

Table I.- Bats (n=47) included in the present study

Season	*S. heathii* (n=23)		*P. pipistrellus* (n=24)	
	Male	**Female**	**Male**	**Female**
Spring (Mar-May)	3	3	5	4
Summer (Jun-Aug)	4	5	3	4
Autumn (Sep-Nov)	5	3	4	4
Winter (Dec-Feb)	---	---	---	---
Total	12	11	12	12

Blood cells counting

Red blood cells (RBC), white blood cells (WBC) and platelets were counted manually by using Neubauer haemacytometer by diluting the blood samples with solutions commercially available for RBC, WBC and platelet counting. The red cell indices, the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated by using the standard formulae (Wintrobe, 1933).

Haemoglobin estimation and haematocrit

Haemoglobin (Hb) was measured by using Sahli's haemometer. For the estimation of hematochrit, plain microcapillary tubes were used. The tubes were centrifuged at 12000 rpm for five minutes in microhaematocrit centrifuge. Haematochrit readings were carried out by using a micro-haematocrit reader (NUVE, NF-048 Turkey).

Statistical analysis

The haematological parameters are shown as Mean±S.E. Overall comparisons of haematological parameters in species were done by using student's t-test. However, seasonal comparisons were done by analysis of variance (ANOVA) following Duncan Multiple Range Test (MSTAT-C, 1991).

RESULTS

Inter-specific variations

P. pipistrellus had significantly (P<0.05) higher mean RBCs, WBCs, platelets, Hb, PCV levels as compared to *S. heathii*. However, mean values of MCV, MCH and MCHC were significantly (P<0.05) higher in *S. heathii* as compared to *P. pipistrellus* (Fig. 1).

Gender based intra-specific variations

Male bats of *S. heathii and P. pipistrellus* had non-significantly (P>0.05) different mean values of RBCs, WBCs, Hb, Platelets, PCV, MCV and MCH as compared to their female counterparts. However, MCH mean values were significantly (p<0.05) higher in female *S. heathii* as compared to male *S. heathii* (Table II).

Seasonal variations in haematological parameters

Bats belonging to both species showed distinct patterns of seasonal variation in various hematological parameters.

RBCs

S. heathii had lowest mean levels (3.94±0.41×106/mm^3) of RBCs in spring season which increased significantly (P<0.05) in summer (9.96±0.46×106/mm^3), and decreased significantly (P<0.05) in autumn (6.51±0.40×106/mm^3). Similarly, in spring, *P. pipistrellus* exhibited mean RBCs count of 8.87±0.39×106/mm^3, which increased significantly (P<0.05) in summer (12.28±0.69×106/mm^3), and decreased significantly (P<0.05) to 10.05±0.40×106/mm^3 in autumn (Table III). Male bats of both species had non-significantly (P<0.05) different mean RBCs indices than female counterparts in all three seasons (Table IV).

WBCs

Both bat species had lowest mean WBCs during spring. *S. heathii* showed 6.58±0.62×103 cells/mm^3 and *P. pipistrellus* had 9.09±0.68×103 cells/mm^3 in spring, which increased (P<0.05) to 15.93±1.15 and 10.51±0.58×103 cells/mm^3 in summer and further increased (P<0.05) to 15.53±1.43 and 19.80±0.76×103 cells/mm^3, respectively in winter (Table III). Male bats of both species had non-significantly (P<0.05) different mean WBCs indices than female counterparts in all three seasons (Table IV).

Platelets

The mean platelet counts were non-significantly (P>0.05) different in both bat species with respect to season. In spring, *S. heathii* had mean platelets estimates of 6.02±1.14, which changed non-significantly (P>0.05) in summer (5.60±0.61) and autumn (6.56±0.91). Similarly, *P. pipistrellus* had mean platelets estimates of 7.38±1.15 in spring, which changed non-significantly (P>0.05) in summer (7.54±1.00l) and autumn (7.64±0.48) (Table III). The mean platelet counts were non-significantly (P>0.05) different in male and female *S. heathii* and *P. pipistrellus* when compared within species by season (Table IV).

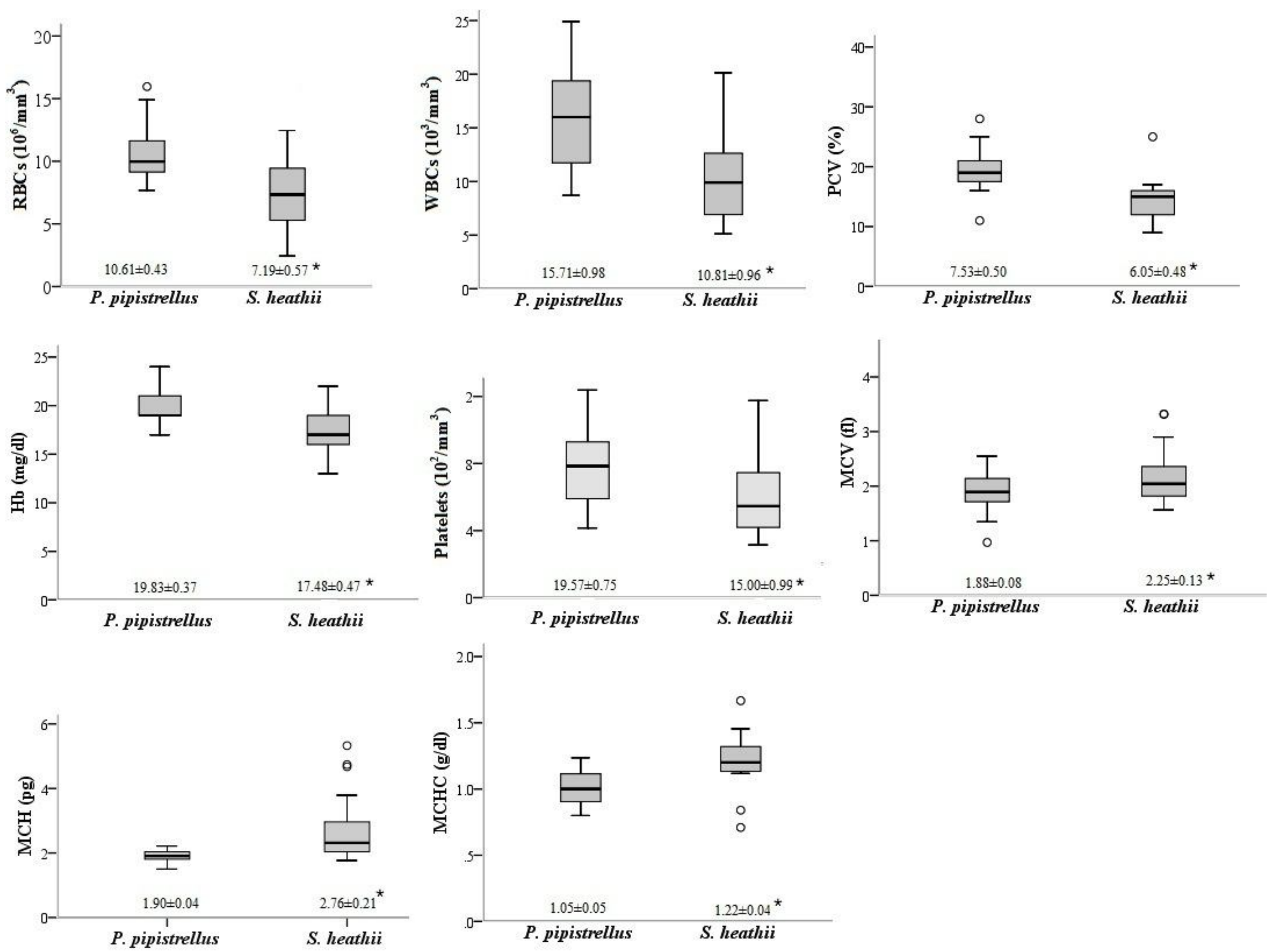

Fig.1. Various haematological parameters of *Scotophilus heathii* and *Pipistrellus pipistrellus*. Mean±S.E. with (*) are significantly different at $p<0.05$.

Haemoglobin

In spring, *S. heathii* had Hb estimates of 15.83 g/dl, changed non-significantly (P>0.05) in summer (19.44±0.50 g/dl) and autumn (16.50±0.33 g/dl). Similarly, *P. pipistrellus* had Hb estimates of 18.17±0.40 g/dl in spring, changed non-significantly (P>0.05) in summer (21.22±0.52 g/dl) and autumn (19.50±0.38 g/dl) (Table III). Furthermore, mean Hb levels were non-significantly (P>0.05) different with respect to gender and seasons (Table IV).

Haematocrit/PCV

In spring, *S. heathii* had lower levels of PCV (11.67±0.84%), which increased significantly (P<0.05) in summer (18.67±1.85%) and then went down significantly (P<0.05) in autumn (13.38±0.62%). Same seasonal variations of PCV had observed in *P. pipistrellus* (Table III). The gender based seasonal variations in PCV were non-significant (p<0.05) when compared within species (Table IV).

MCV

MCV levels in *S. heathii* during spring were highest (3.08±0.28 fl) which decreased significantly (P<0.05) in summer (1.85±0.10 fl) and changed non-significantly (P>0.05) in autumn (2.07±0.04 fl). A non-significant (P>0.05) difference was observed in MCV of *P. pipistrellus* during different seasons (Table III). Furthermore, mean MCV estimates were non-significantly (P>0.05) different in male and female *S. heathii* and *P. pipistrellus* when compared within species in different seasons (Table IV).

Table II.- Comparison of haematological parameters (Mean±S.E.) of *S. heathii* and *P. pipistrellus*.

	S. heathii		*P. pipistrellus*	
	Male (n=12)	**Female (n=11)**	**Male (n=12)**	**Female (n=12)**
RBCs (10^6/mm^3)	7.62±0.78	6.72±0.86	11.30±0.69	9.86±0.43
WBCs (10^3/mm^3)	10.85±1.51	10.78±1.21	15.89±1.56	15.50±1.23
Platelets (10^2/mm^3)	7.02±0.68	4.98±0.53	7.80±0.70	7.24±0.74
Hb (mg/dl)	17.67±0.63	17.27±0.71	20.50±0.53	19.09±0.41
PCV (%)	16.25±1.69	13.64±0.85	19.58±1.36	19.55±0.59
MCV (fl)	2.20±0.14	2.30±0.24	1.76±0.11	2.02±0.10
MCH (pg)	2.51±0.19	3.03±0.38*	1.85±0.06	1.95±0.04
MCHC (g/dl)	1.15±0.06	1.29±0.05	1.10±0.08	0.99±0.04

* Significantly different at P<0.05.

Table III.- Seasonal variations of haematological parameters (Mean±S.E.) of *S. heathii* and *P. Pipistrellus.*

	S. heathii			*P. pipistrellus*		
	Spring	**Summer**	**Autumn**	**Spring**	**Summer**	**Autumn**
RBCs (10^6/mm^3)	3.94±0.41^{c}	9.96±0.46^{a}	6.51±0.40^{b}	8.87±0.39^{Y}	12.28±0.69^{X}	10.05±0.40^{Y}
WBCs (10^3/mm^3)	6.58±0.62	9.09±0.68	15.93±1.15	10.51±0.58	15.53±1.43	19.80±0.76
Platelets (10^2/mm^3)	6.02±1.14	5.60±0.61	6.56±0.91	7.38±1.15	7.54±1.00	7.64±0.48
Hb (mg/dl)	15.83±0.95	19.44±0.50	16.50±0.33	18.17±0.40	21.22±0.52	19.50±0.38
PCV (%)	11.67±0.84^{b}	18.67±1.85^{a}	13.38±0.62^{b}	17.17±0.48^{y}	21.00±1.18^{x}	19.75±1.47^{xy}
MCV (fl)	3.08±0.28^{a}	1.85±0.10^{b}	2.07±0.04^{b}	1.95±0.10	1.73±0.10	2.00±0.18
MCH (pg)	4.19±0.36^{a}	1.97±0.04^{c}	2.58±0.11^{b}	2.06±0.05^{x}	1.75±0.05^{y}	1.95±0.04^{xy}
MCHC (g/dl)	1.37±0.08^{a}	1.09±0.06^{b}	1.25±0.04^{ab}	1.06±0.04^{x}	1.03±0.05^{y}	1.05±0.13b^{y}

Significantly different at P<0.05; Values sharing same letters are non-significantly (P>0.05) different.

Table IV.- Gender based seasonal variations of haematological parameters (Mean±S.E.) of *Scotophilus heathii* and *Pipistrellus pipistrellus.*

		Scotophilus heathil			*Pipistrellus pipistrellus*		
	Gender	**Spring**	**Summer**	**Autumn**	**Apring**	**Summar**	**Autumn**
RBCs (10^6/mm^3)	Male	4.59±0.41	10.55±0.97	7.10±0.45	9.15±0.43	13.83±1.05	10.57±0.51
	Female	3.29±0.52	9.49±0.26	5.52±0.23	8.59±0.70	11.03±0.43	9.18±0.19
WBCs (10^3/mm^3)	Male	6.57±0.39^{b}	7.44±0.93^{b}	16.14±1.55^{a}	9.42±0.44d^{z}	16.64±3.12^{wx}	19.17±0.98^{wv}
	Female	6.59±1.32^{b}	10.42±0.42^{b}	15.58±2.06^{a}	11.59±0.55^{y}	14.64±1.02^{w}	20.86±1.12^{v}
Platelets (10^2/mm^3)	Male	6.68±2.12	6.89±0.49	7.34±1.24	7.98±1.02	7.60±2.00	7.85±0.67
	Female	5.35±1.24	4.57±0.78	5.28±1.18	6.78±2.29	7.50±1.10	7.28±0.72
Haemoglobin (mg/dl)	Male	15.67±0.33	20.00±1.08	17.00±0.32	18.67±0.33	22.50±0.65	20.00±0.45
	Female	16.00±2.08	19.00±0.32	15.67±0.33	17.67±0.67	20.20±0.37	18.67±0.33
Haematocrit (%)	Male	12.33±0.88	21.75±3.82	14.20±0.73	16.33±0.33	23.25±2.06	18.60±2.25
	Female	11.00±1.53	16.20±0.37	12.00±0.58	18.00±0.58	19.20±0.80	21.67±0.67
MCV (fl)	Male	2.75±0.38	2.02±0.20	2.01±0.03	1.79±0.05	1.71±0.19	1.78±0.24
	Female	3.41±0.38	1.71±0.02	2.17±0.03	2.12±0.13	1.75±0.11	2.36±0.10
MCH (pg)	Male	3.46±0.25^{b}	1.92±0.08^{e}	2.43±0.13^{d}	2.05±0.07^{xy}	1.65±0.09^{z}	1.90±0.05^{xy}
	Female	4.91±0.21^{a}	2.01±0.03^{e}	2.84±0.09^{c}	2.07±0.09^{x}	1.84±0.04^{yz}	2.03±0.04^{xy}
MCHC (g/dl)	Male	1.28±0.09	0.98±0.13	1.21±0.05	1.14±0.02	1.65±0.09	1.16±0.19
	Female	1.47±0.11	1.17±0.01	1.31±0.04	0.98±0.02	1.06±0.06	0.86±0.02

Values sharing same letters are non-significantly (P>0.05) different.

MCH

S. heathii and *P. pipistrellus* bats had high levels of MCH in spring (4.19±0.36 and 2.06±0.05 pg, respectively) which significantly (P<0.05) decreased (1.97±0.04 and 1.75±0.05, respectively) in summer. In autumn, the values of MCH increased significantly (P<0.05) in *S. heathii* (2.58±0.11 pg) and changed non-significantly (P>0.05) in *P. pipistrellus* (1.95±0.04 pg) (Table III). Male and female *S. heathii* showed significant (P<0.05) difference in MCH in spring and autumn and non-significant (P>0.05) difference in the summer (Table IV).

MCHC

In spring, *S. heathii* had MCHC estimates of 1.37±0.08 g/dl, which decreased significantly (P<0.05) in summer (1.09±0.06 g/dl), but remain same (P>0.05) in autumn (1.25±0.04 g/dl). *P. pipistrellus* had non-significant (P>0.05) differences in MCHC in different seasons (Table III). Furthermore, MCHC estimates were non-significantly (P>0.05) different in male and female *S. heathii* and *P. pipistrellus* when compared within species in different seasons (Table IV).

Correlation of haematological parameters within gender and species

The haematological parameters of male bats of both species did not show any correlation in spring. However, a significant (p<0.05) positive correlation (r=0.98) between PCV and Hb, while a significant (p<0.05) negative correlation (r=-0.89) between MCHC and Hb were observed in the female *S. heathii*. Furthermore, in the female *S. heathii*, PCV was correlated (r=-0.98) with MCHC in spring. However, in female *P. pipistrellus* only PCV is significantly (p<0.05) correlated (r=-0.97) with MCH.

In summer, RBCs counts were significantly (p<0.05) correlated with Hb (r=0.98), PCV (r=0.86), MCV (r=-0.99) and MCH (r=-0.92) in male *S. heathii*. Similarly, male *P. pipistrellus* had RBCs counts correlated with MCV (r=-0.983), MCH (r=-0.955) and MCHC (r=0.988). In male *S. heathii*, Hb levels were positively correlated with PCV (r=0.97) and negatively correlated with MCV (r=-0.92) and MCH (r=-0.99). Furthermore, PCV was correlated with MCV (r=-0.95) and MCH (r=-0.98) in male *S. heathii*. Similarly, in male *P. pipistrellus*, the PCV was correlated with MCV (r=0.98), MCH (r=0.97), and MCHC (r=-0.96) in summer. In female *P. pipistrellus*, RBCs are correlated with Hb (r=0.987) and MCH (r=-0.98) and Hb is correlated (r=-0.94) with MCH in summer.

In autumn, the male *S. heathii* had RBCs counts positively correlated with Hb (r=0.99), PCV (r=0.98), MCV (r=0.94) and negatively correlated with MCH (r=-0.98) and MCHC (r=-0.98). Furthermore, Hb was positively correlated with PCV (r=0.98) and MCV (r=0.94) and negatively correlated with MCH (r=-0.98) and MCHC (r=-0.96) in male *S. heathii*. In male *S. heathii*, the PCV was also positively correlated with MCV (r=0.98) and negatively correlated with MCH (r=-0.95) and MCHC (r=-0.97) in autumn. Similarly, in male *P. pipistrellus* RBCs counts were correlated with Hb (r=0. 92) and MCH (r=-0.96), and PCV was correlated (r=-0.97) with MCHC. In female *P. pipistrellus* only PCV and MCH were correlated (r=0.99) in autumn.

DISCUSSION

Seasonal hematological studies are required to reveal the physiological seasonal adaptations which also help in differential diagnosis of normal and pathological conditions (Gulland and Hawkey, 1990; Campbell, 1992; Dawson and Bortolotti, 1997; Heard and Whittier, 1997). The blood composition may vary with various internal and external factors *i.e*. age, gender, reproductive state, season, diurnal rhythm, pollution, food quality and availability (Westhuyzen, 1978; Hellgren *et al*., 1988; Wolkers *et al*., 1994; Minemastue *et al*., 1995). In addition to the seasonal changes, the blood profile changes due to capturing, handling and sampling stresses (Widmaier and Kunz, 1993; Koopman *et al*., 1995).

The oxygen carrying capacity of blood is dependent on the counts of erythrocytes increase during summer season to support the heightened activity of the bats (Arevalo *et al*., 1987; Korine *et al*., 1999). During the period of the lesser activity, bats do not feed or feed less which may result in a decrease of metabolic rates and RBCs. It has been observed that the concentrations of the blood components change when an animal gets exposed to low temperature (cold conditions) and low quality of food (Nieminen, 1979; Hellgren *et al*., 1988; Alfaro *et al*., 1994; DelGiudice *et al*., 1994; Wolkers *et al*., 1994).

WBCs levels were found lower during spring and summer seasons as compare to autumn because bats are involved actively in reproduction, and the resources required may be used more for reproductive success than survival through immunity (Roff, 1992; Stearns, 1992; Sheldon and Verhulst, 1996; Lochmiller and Deerenberg, 2000; Norris and Evans, 2000; Schmid-Hempel and Ebert, 2003; Harshman and Zera, 2006). Furthermore, reproductive success of animals depends on the survival of gametes, while, the autoimmunity is one of a major threats to gametes. In order to ensure gametes survival, immunity of bats may be down regulated during the reproductive season (Folstad and Skarstein, 1997; Kortet *et al*., 2004; Skau and Folstad, 2004). The higher WBCs counts may indicate presence of stress or infection (Pehlivanoglu *et al*., 2001).

Although, the present study reported almost similar levels of platelets in different seasons, but previous studies in human reported that in addition to genetic factors, gender, age and seasonal factors may regulate platelet counts. In contrast to our results, higher counts were observed in human females than in males (Biino *et al*., 2011). Seasonal variations in human platelet counts were observed with a peak during autumn and winter (Buckley *et al*., 2000).

Bats have high haematocrit and haemoglobin levels than terrestrial mammals (Lewis, 1977; Jurgens *et al*., 1981; Wightman *et al*., 1987; Arevalo *et al*., 1987, 1992; Viljoen *et al*., 1997). In contrast to our results, previous studies reported that at the end of the resting phase (winter season) the haemoglobin and haematocrit levels of bats were higher as compared to the end of the activity phase (summer season) during which the values of both these parameters were low (Korine and Arad, 1993). However, high blood-oxygen requirement during activity is responsible for high haemoglobin concentrations and haematocrit values in small mammals with high metabolic rates (Arevalo *et al*., 1992).

The increase in MCV suggests an increase in the number of immature erythrocytes (Lurie, 1993; Kim *et al*., 2002). Haematocrit amount and MCV increased during the summer months and decreased during winter and spring months that is related to variations in activity of bats in these seasons (Arevalo *et al*., 1992; Korine *et al*., 1999). The higher MCH and MCHC values in spring may be a sign of an increase in erythropoietic activity of the bats related to increase in temperature and photoperiod leading to increase in erythrocyte count, haemoglobin and hematocrit (Rewkiewiccz, 1975). Furthermore, a positive correlation between PCV and Hb showed a constant Hb concentration in the RBCs. The positive correlation of RBCs with Hb and PCV, and negative correlation with MCV and MCH indicates the reduced size of RBCs with increasing number of cells. The positive correlation between RBCs, Hb and PCV showed that increasing cell numbers also increase Hb and PCV (Hardig and Hoglund, 1983).

The bats (*Scotophilus healthii* and *Pipistrellus pipistrellus*) showed interspecific and gender based intraspecific haematological variations. The seasonal fluctuations in haematological parameters were also observed in the bats studied. *P. pipistrellus* had higher counts of RBCs, WBCs, platelets, Hb and PCV as compared to *S. heathii*. The higher levels of RBCs, Hb and PCV of the bat species were associated with a higher activity season, *i.e.*, summer and higher levels of WBCs were associated with less activity season, *i.e.* autumn.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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