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Hematology and biochemical assessment of healthy Night Herons (*Nycticorax nycticorax*)

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Abstract
The night heron (*Nycticorax nycticorax*) is a medium-sized bird belongs to the family Ardeidae along with other members like egrets. In the present study, haematobiochemical parameters of night herons were evaluated on 10 clinically healthy night heron at Jabalpur forest division (Madhya Pradesh). The birds were rescued from near the residential areas and keep in the Outdoor patient department (OPD) of School of Wildlife Forensic and Health, NDVSU, Jabalpur for observation. Blood were collected from wing vein and immediately dispensed in two sterile vacutainers, one with EDTA and the other was in clot activator (Peerless Biotech Pvt. Ltd). Plasma was separated and major biochemical parameters were analyzed. The Mean±SE values for the parameters were: Red blood count (RBC) 2.49±0.08 10^6/µl, White blood count (WBC) 172.24±3.83 10^3/µl, hemoglobin (Hb) 16.79±0.64 g/dl, Packed cell volume (PCV) 36.50±1.34%, MCH 65.51±2.11 pg, MCV 146.18±4.92 fl, MCHC 49.45±0.80 g/l, platelets 204.09±15.2310^3/µl, heterophils 62.73±1.09%, lymphocytes 32.45±1.03%, monocytes 1.91±0.25%, eosinophils 2.00±0.23% and heterophil: lymphocyte 1.92±0.08. The biochemical parameters analyzed: TP 4.32±0.02 g/dl, SGOT/AST 73.27±0.77 µ/l, UA 5.15±0.04 mg/dl, these haemato-biochemical parameters can act as reference values for future analysis involving health screening of night heron.

Keywords: Night heron, healthy, blood, hematology, biochemical

Introduction
The night heron (*Nycticorax nycticorax*) is a medium-sized bird belongs to the family Ardeidae along with other members like egrets (Yogamaya and Prafulla 2016) [9]. The members are mostly residents or semi-migratory in nature and are one of the widely distributed populous avian species with top feeding positions to be found in most of the wetland ecosystems. They are very noisy birds in their nesting colonies. These birds stand still at the water's edge and wait to ambush prey, mainly at night or early morning. The breeding habitat is fresh and salt-water wetlands throughout much of the world (Hahn *et al*., 2006) [10]. India is home to nine heron species, many of which are found across the country. While most of the heron species in India are designated as Least Concern from a conservation point of view, degradation of wetlands and pollution of water bodies are threats that impact local populations. The fact that heron populations and threats to them are not well-studied further confounds conservation of the species. In 2020, Nature Conservation Foundation conducted the first-ever survey of the critically endangered White-bellied Herons across 81 sites in northeast India. The study found that various anthropogenic activities like sand/gravel mining, hydropower projects and garbage dumping impacted the sighting of the bird in specific regions. More importantly, the study highlighted that protection of herons needs more focused research and greater awareness among the public. This revelation comes to us at a time when we have lost a significant percentage of wetlands. According to the Wetland International South Asia, India has lost a third of its wetlands to pollution, developmental and agricultural activities in the last four decades. Herons are among the many birds that depend on these water bodies, which make it imperative that we look at the species in isolation to devise better conservation practices suited to them. In the 19th century, this bird was extensively hunted for its aesthetic values, since than continued to be hunting of this bird. The status of the population of heron has been proved to be an important factor for the assessment of overall environmental wellbeing (Blus *et al*., 1997) [1]. Along with other assessment methods like habitat and population studies, health assessment tool like haematological analysis is also important for wildlife studies. However, being categorized as least concerned, the members of this family perhaps fail to draw attention in this respect and thus, very few literature is available on hematology of herons.

Most of the studies conducted are on other popular species having either economic importance or having a threatened existence (Villegas *et al*., 2004) [8]. Moreover, no proper haematological data are on record for grey herons except a few (Newman *et al*., 2007) [5]. This study has been performed on 33 night heron of Jabalpur forest division for generate a base line values of hematology and serum biochemistry of night heron (*Nycticorax nycticorax*).

Method and materials

10 healthy night herons were prior to release in natural habitat brought to OPD of School of Wildlife Forensic and health, NDVSU, Jabalpur by Madhya Pradesh Forest Department from residential areas for clinical observation. They were held in cages. They were allowed to acclimatize for few hours and were feed small fishes and water was provided ad libitum. Food was withheld for 6 hours before samples of blood were collected. During the acclimatization period, the herons were closely observed for clinical abnormalities and were found to be normal.

Birds were handled by using physical restraint method (protective gloves) and approximately 2.5 ml blood was collected from the wing vein by using 26 gauge needle and 3 ml disposable syringes and immediately stored in a sterile vacutainer containing EDTA (ethylene diamine tetraacetic acid) as an anticoagulant (Bio *in-vitro* Diagnostics Pvt. Ltd., Waghodia) in ice box. The blood smears were prepared soon after the blood collection and stained with Romanowsky stain for examination of blood protozoa as well as for carrying out differential leukocyte count for interpretation of infectious manifestations. The blood samples were taken to the laboratory for further study within 24 hours of sample collection following standard procedure (Campbell *et al*., 2001) [2]. Haemoglobin concentration was estimated using Sahli's Haemoglobinometer and PCV was estimated by microhaematocrit method running the sample at 2000 rpm in centrifuge for 15 minutes. Total RBC, WBC and thrombocyte counting were done with the help of Neubaurer's haemocytometer. The mean corpuscular volume (MCV), mean haemoglobin concentration (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using standard formulae (Thrall *et al*., 2012) [7]. For the DLC, blood smears were stained in Romanovsky stain (Leishman's Stain). WBCs were counted and classified according to their staining and morphologic properties. Biochemicals parameters such as total protein, aspartate aminotransferase (AST) for liver function, uric acid (UA) for kidney function and alkaline phosphatase (AP) to correlate the stress factors were determined using standard kits. The Descriptive statistics (mean, standard deviation, standard error and range) analysis of recovered baseline data was tested as per Snedecor and Cohran (1994) [6] and used SPSS software 13.0 version for analysis of values for interpretation and conclusion.

Result and Discussion

Hematology and plasma/serum biochemical parameters are important in animal health evaluations because they specifically assess the function and condition of organs, identify clinical pathology and potential exposure to diseases. The hematological analysis of avian species depends on various factors like age, species, hormonal, physiological, capture methods, sample collection techniques, storage and handling of samples, and methods of analysis may prevent accurate interpretations of results (Low *et al*., 2006) [11]. The haematological parameters including erythrocytes, lymphocytes, haemoglobin of night heron found within the range similar to blood profile of black crowned night herons *(Nycticorax nycticorax*) (Thrall *et al*., 2012) [7] which are under the normal reference range (Table 01). This study indicates a slightly lower PCV, lymphocyte value but higher value of MCV, heterophils, MCH, RBC, WBC and MCHC for night heron in comparison to that reported by Thrall *et al*. (2012) [7]. Lower and higher values of hematological parameters may be due to reproductive and physiological condition of bird and environmental conditions like temperature, availability of food etc. (Etim *et al*., 2014) [3].

WBC indicates the function of immune system and an organism's response to infection (Hylton *et al*., 2006) [12]. It also indicates the balance between supply and the need for leukocytes to defend the body against pathogens and other foreign materials introduced into the body. Increased WBC counts are results of exposure to contaminants or microorganisms like bacteria, parasites, viruses, fungi or parasites. In our study normal WBC range is slightly increased with the comparison of Thrall *et al*. (2012) [7], it may be due to exposure to contaminants, although parasitic infection was not found in any of the bird. Prey diets included a variety of fish and there is a close relationship between food intake and the bioaccumulation of contaminants (Gray, 2002) [13]. 3 herons showed higher range of PCV, the reason behind this may be loss of fluid or dehydration, although rest of the herons was having normal range of PCV. The H:L ratio has also been observed as an indicator of stress, immune system function and long-term changes in the environment (Newman *et al*., 2007) [5]. An increased heterophils count due to stress or exposure to bacteria, parasites, viruses fungi, or contaminants is likely the cause of the high H:L ratio observed in herons. Higher activity of AST has been allied with hepatic disease in caged birds and muscle damage and muscular dystrophy in domestic fowl (Newman *et al*., 2004) [14]. Here are some values increase might be due to lack of activity. Other parameters were seen under normal reference range.

Table 1: Hematological and biochemical parameters of Night heron (*Nycticorax nycticorax*)

S. No.	Parameters	Mean±SE (N=10)	Standard deviation (N=10)	Range (N=10)	*Reference values
1.	WBC (10^3/µl)	17.24±3.83	12.71	14-18	8-14
2.	RBC (10^6/µl)	2.49±0.08	62.68	2.00-2.85	2-4
3.	HB (g/dl)	14.79±0.64	2.14	13.9-16.5	12-15
4.	PCV (%)	33.50±1.34	4.44	36-40	38–42
5.	MCV (fl)	146.18±4.92	16.33	127-185	120-142
6.	MCH (pg)	65.51±2.11	7.002	50-75	41-49
7.	MCHC (g/l)	49.45±0.80	2.68	47-54	32-45
8.	PLT (10^3/µl)	204.09±15.23	50.52	133-260	188-246

9.	Heterophils (%)	62.73±1.09	3.63	58-67	61-67
10.	Lymphocytes (%)	32.45±1.03	3.41	28-38	32-35
11.	Monocytes (%)	1.91±0.25	0.83	1-3	0–7
12.	Eosinophils (%)	2.00±0.23	0.77	1-5	2-4
13.	H:L	1.92±0.08	0.29	1.7-2.4	1-1.5
14.	SGOT (µ/l)	73.27±0.77	8.52	58-84	22–73
15.	Uric acid (mg/dl)	16.3±0.8	0.50	4.5-22.6	2.6–37.5
16.	Total protein (g/dl)	4.32±0.02	0.24	3.8-4.6	0.2–5.0

***(**Thrall *et al*., 2012) [7]

Conclusion

Herons are an important group of birds in any wetland ecosystem. They belong to tertiary trophic level in an ecosystem and as the same health of their members may easily reflect any alteration in biotic or abiotic components of the ecosystem. Thus, they may play a key role in studying the wellbeing of their environment. This study is an effort to record haematological data of night herons located from the urban areas may be useful for future reference in ecological, wildlife as well as veterinary purposes.

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