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A Ramakrishnan
M.V.Sc., Scholar, Department of Wildlife Science, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, India

M Palanivelrajan
Assistant Professor, Department of Wildlife Science, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, India

M Thangapadiyan
Assistant Professor, Department of Veterinary Pathology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, India

D Sumathi
Assistant Professor, Department of Veterinary Clinical Medicine, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, India

K Senthilkumar
Assistant Professor and Head i/c, Department of Wildlife Science, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, India

Correspondence
A Ramakrishnan
M.V.Sc., Scholar, Department of Wildlife Science, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, India

Haematology analysis of rescued olive Ridley Sea Turtles (*Lepidochelys olivacea*)

A Ramakrishnan, M Palanivelrajan, M Thangapadiyan, D Sumathi and K Senthilkumar

Abstract
The olive Ridley Sea Turtle are smallest and most abundant of all species of sea turtle found in warm and tropical waters, primarily in the Pacific and Indian Oceans. The research work was carried out during the period from September, 2017 to April, 2018, on stranded Olive Ridley Sea Turtles (*Lepidochelys olivacea*) that were stranded off on East Coast Beach along Chennai coast and maintained at the rescue center of Trust for Environmental and Education (TREE) foundation Tree Foundation, Vettuvankeni, Chennai, Tamil Nadu. The blood samples are taken from Olive Ridley Sea Turtles that are at the rescue and rehabilitation centers. The blood cells which were identified by differential count are mature erythrocytes, heterophils, eosinophils, lymphocytes, monocytes and thrombocyte. The clinical parameters such as Haemoglobin (Hb) was (85.71±11.96), Packed Cell Volume (PCV) was (0.29±0.04), White Blood Cells (WBC) was (5.78±0.85) and Red Blood Cells (RBC) was (0.60±0.06) evaluated for the rescued Sea Turtles. These Sea Turtle undergoing rehabilitation gives the critical parameters which are intended for health evaluations of sea turtle in rescue centers.

Keywords: Olive Ridley Sea Turtle, blood cell, morphology

Introduction
There are seven species of marine Sea Turtles globally which are listed on the IUCN Red List [11]. Marine turtles have swum in the world's oceans for over 100 million years. Olive Ridley Sea Turtles are scheduled as vulnerable under International Union for Conservation of Nature and Natural Resources (IUCN) globally [21]. The common Sea Turtles of Tamil Nadu include Leatherback Sea Turtle (*Dermochelys coriacea*), Hawksbill Sea Turtle (*Eretmochelys imbricata*) and Olive Ridley Sea Turtle. Among these turtle species the most common species which are stranded commonly are Olive Ridley species of Turtles [5]. The Olive Ridley Sea Turtle *(Lepidochelys olivacea)* is one of the smallest species of sea turtle in the world (80cm). They are the most numerous and widely distributed turtles species in the Indian sub-continent. India is one of the few places in the world where olive Ridley gather in the thousands to nest en masse [3]. The haematology and morphology of blood cells have been established for most of the Sea Turtle species but there is lacking of established data for Olive Ridley Sea Turtles and Flatback Turtles (*Natator depressus*) [20]. Here, this study was carried out to establish a base data on haematology of Olive Ridley Sea Turtles under rehabilitation.

2. Materials and Methods
Seven Olive Ridley Sea Turtles that were stranded off in the East Coast Beach along Chennai coast and maintained at the rescue center of Trust for Environmental and Education (TREE) foundation Tree Foundation, Vettuvankeni, Chennai, were used in this study. The study periods were from September, 2017 to April, 2018. The age of rescued turtles had a time period starting from recently rescued to oldest rescued Sea Turtle. Turtles were monitored regularly both in-water along and out-water examination to assess the health condition.

Fig 1: Site for Blood collection (Dorsal cervical sinus)

In Olive Ridley Sea Turtles venipuncture is a blind technique, because the blood vessels are not palpable. The collection was done as 1 ml of blood per 100 grams of body weight (1% of total body weight) using 22G needle. The common blood collection site was dorsal cervical sinuses. The animal was restrained in dorsal recumbency with its head over the edge of a table. The head was held extended from the body and tipped in slight ventroflexion and then, the needle was inserted behind the occipital, in a caudal direction, at an angle of 30° and then the blood was collected (Fig 1). Blood samples were drawn in Lithium Heparin coated tubes for haematology which was stored at 4^0. The blood smears were fixed in methanol and were used for blood parasite examination and differential leukocyte counts after staining with Leishman-Giemsa stain [9]. The blood smears were examined under an oil immersion microscope (100 x) objective. The maximum and minimum diameter of blood cells (15 erythrocytes and 15 leucocytes) and their nuclei were measured for each smear with a calibrated ocular micrometer. Blood cell and nuclear areas were calculated according to the following formula: (length ×width×)/4 [18]. The statistical analyses of the data for various parameters of sea turtles were carried out as by using student's t-test.

3. Results

Mean haematologial values of Olive Ridley sea Turtles are shown below in Table 1.

Table 1: Mean (S.D) values of haematology for Olive Ridley Sea Turtle (n=7)

S. No.	Parameters		Units	Mean (S.D)	S.E	Median
1	Haemoglobin (Hb)		g/l	85.71(11.968)	4.52	85.00
2	Packed Cell Volume (PCV)		l/l	0.2985(0.043)	0.016	0.28
3	Red Blood Cells (RBC)		10^6/µl	0.60(0.064)	0.02	0.59
4	White Blood Cells (WBC)		10^3/µl	5.78(0.852)	0.32	0.54
5	Differential Count (DC)	Heterophils	10^3/µl	60.85(6.011)	2.27	60.00
		Lymphocytes	10^3/µl	35.71(6.047)	2.28	36.00
		Monocytes	10^3/µl	3.28(1.112)	0.42	3.00
		Eosinophils	10^3/µl	1.42(0.534)	0.20	1.00
		Basophils	10^3/µl	0	0	0

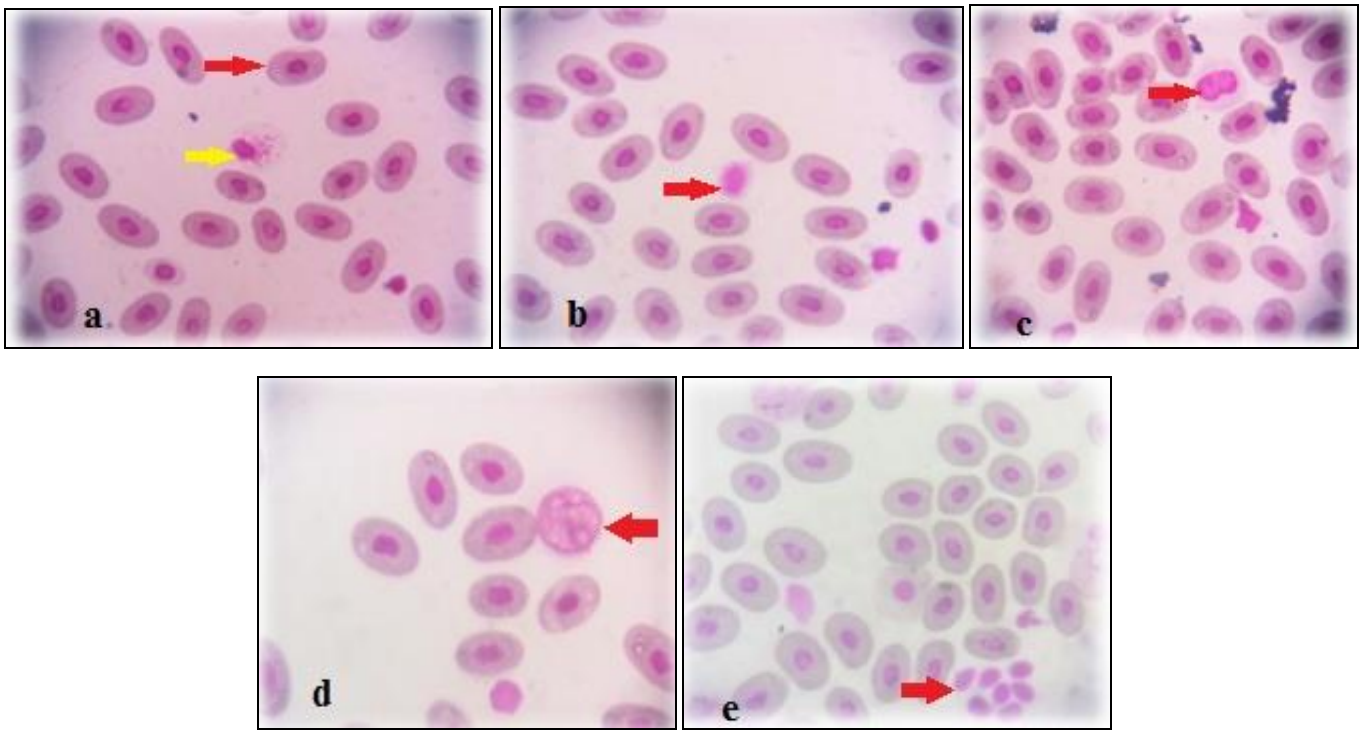

Fig 2: Photomicrographs of blood cells from olive Ridley turtles (*Lepidochelys olivacea*) stained with Leishman–Giemsa. a) A mature erythrocyte *(arrow*) with basophilic intracytoplasmic inclusions and eosinophil (*arrow head*) b) Lymphocyte c) Monocyte d) Heterophils e) Thrombocyte

The peripheral blood of the Olive Ridley Sea Turtles was taken and the following blood cell was identified as erythrocyte, heterophils, eosinophils, lymphocyte, monocyte and thrombocyte (Fig 2). Erythrocytes were elongated oval cells with centrally located spherical shaped nuclei and the chromatin was dense purple colored. Heterophils are cells that

were numerous in number which are in round to oval shaped that contained numerous azurophilic granules in the cytoplasm. Lymphocytes were large round cells and these cells having highest nucleus: cytoplasm (N: C) ratio of all the leukocytes with nucleus containing large round nuclei predominant eosinophilc cytoplasm. Monocyte cells were round or in amoeboid shape while the nucleus has variable from ranging from round, oval and monocyte nucleus chromatin was less condensed on comparison with lymphocytes. Thrombocytes were oval shaped cells, some of the cells are spherical to round shaped contained oval to round nuclei containing coarse chromatin.

4. Discussion

The rescued Sea Turtles provide a valuable data for conservation of the Olive Ridley species since, this species of the turtles were listed as Vulnerable by International Union for Conservation of Nature and Natural resources (IUCN). The Olive Ridley Sea Turtles are legally protected under Schedule I of the Wildlife Protection Act, 1972 and Appendix I of the CITES Convention which prohibits trading of turtle products [17].

The Packed Cell Volume (PCV), Haemoglobin (Hb), Red blood cells (erythrocyte) counts and White blood cells (leukocyte) counts were found to be highly similar with findings of the earlier studies [11, 14]. The erythrocytes observed were largest cell among the Sea Turtle blood cells [15].

The differential leukocyte count revealed that among leukocytes heterophils were the predominant cells which are followed lymphocytes and monocytes. This result is similar to findings of previous studies [1, 2, 7, 8, 14]. The mean values obtained for heterophils, lymphocytes, eosinophils were comparable to those found by previous studies [16]. However, there was appreciable increase with the count of monocytes, which was in similar to the previous studies in reptiles. This could be due to chronic antigenic stimulation, chronic inflammation, bacterial or parasitic diseases [10]. The thrombocyte values which were cited in the study were in concurrence of previous studies [1, 6, 14].

The morphological and Ultrastructure of the blood cells identified were similar to that of previous studies [1, 4, 12, 13, 19, 20]. Futherwork, on correlation of cell morphology with disease conditions will enable the clinicians to treat chelonians in a better and precise way.

5. Conclusion

This study has provided reference haematological intervals which could be highly useful in assessing the health condition of the turtles in rescue and rehabilitation centers for future conservation aspects. The information from working reference intervals can be helpful in sea turtles for identifying various abnormalities which will help to conserve the rescued and rehabilitated sea turtles effectively. This will open scope for the clinicians for furnishing basic haematology information of *L. olivacea*. So the quality of life of rescued sea turtles will be improved in terms of conservation of Chelonian species.

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7. References

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