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Haemato-biochemical alterations of fractured painted storks (*Mycteria leucocephala*) due to gale in Koonthankulam bird sanctuary, Tamilnadu

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Abstract
A strong gale had uprooted many trees in koonthankulam bird sanctuary in Tirunelveli district during the Month of April, 2019. Around 133 more birds were injured, 68 were killed and 13 were critically injured with fractures in the wings and legs. The 13 injured birds were brought to the Veterinary clinical complex, VCRI, Tirunelveli for further investigation and treatment. On clinical examination, the birds were injured badly and fractures were identified at various sites like femur, tibiotarsus, tarsometatarsal and humerus region. The blood sample were collected from the wing vein and analysed for the haematobiochemical alterations. The haematological values revealed significant changes like reduction in values of haemoglobin, packed cell volume, Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). The biochemical values revealed abnormal increase in alkaline phosphatase enzyme (ALP) and aspartate aminotransferase (AST) and creatine phosphokinase (Cpk). The changes in haematobiochemical values of the painted storks with fracture were presented and discussed.

Keywords: Gale, Koonthankulam bird sanctuary, painted stork, fracture, haematobiochemical alterations

Introduction
Koonthankulam Bird Sanctuary is well-known for its exciting congregation of water birds, located in the latitude of 8.58102°N 77.76123°E, Tirunelveli district along the banks of the river Thamirabarani, Tamil Nadu. It is having a protected area of 1.2933 km^2 (0.4993 sq mi) and declared as a bird sanctuary in 1994. This is the largest reserve for breeding water birds in South India. More than 43 species of resident and migratory water birds visit here every year. More than 100,000 migratory birds start coming by December and return back to their homes by June or July when their offsprings are mature enough to fly.

Painted Stork (*Mycteria leucocephala*) is a large and brightly colored water bird (wader) of Indian sub- continent, South-West China and parts of South East Asia and can be found on fresh lakes and marshes [5]. Its belongs to the animal kingdom is phylum Chrodata, class Aves, order Ciconiiformes, family Ciconiidae and genus Mycteria. The family ciconiidae includes 19 species in the world, but only 11 species existing in Asia [8]. The painted stork measures less than a meter in its total height of the bird (56 to 58 inches.). The bird is white in color with black markings and fine pink on the lower back, black and white lesser than wing cover and a black band across the breast [5]. Painted Stork had an orange-red head, large heavy yellow beak and pink long legs. Both the sex looks alike. They feed mainly fishes and frogs, which they get in the shallow water [10]. Its breeding season is during the monsoon in north India and November to February in South India. Maximum of 3 - 5 eggs, the nest are on the trees near the water and nesting period 7 - 18 weeks. Both the sex takes part in incubating and rearing the chicks [3].

Haemato biochemical analysis in avian species is a useful tool for the diagnosis of avian diseases [2], as the blood represents a means of assessing clinical and nutritional health status of animals and birds. At present this birds recognized as an endangered species. The purpose of the study was to evaluate the haemato biochemical alterations in the painted stork with fracture and muscle injury at various sites.

Materials and Methods

During the last week of April' 2019, a strong gale had uprooted many trees in koonthankulam bird sanctuary of Tirunelveli district. In that gale, too many species of birds were affected and died due to stamping of trees. A total of 133 birds were injured, 68 were killed and 13 young to adult painted storks were critically injured with fractures in the wings and legs. and were brought to the Veterinary clinical complex, VCRI, Tirunelveli for treatment. On thorough clinical examination, fractures at several sites and severe muscle injury were identified in all the birds. The whole blood samples of about 4 ml were collected from the wing vein (Fig. 1**)** using 24 gauge, 1.5 inches needles and half of the blood was transferred into EDTA containing vacutainer for haematological analysis and the rest of the half into the clot activator vial for determination of serum biochemical values [7] using the semi-automated biochemical analyzer (Lab mate) by spectrophometerically using standard diagnostic kits. The blood smear were prepared and fixed in methanol and stained with Leishman-Giemsa stain and then examined for the differential leucocyte count. The haematological parameters like haemoglobin concentration, red blood cell and packed cell volume were determined by cyanmethemoglobin, Natt and Herrick's method and microhaematocrit method method respectively [2, 9].

Results and Discussion

The fractures were reported at different sites for individual birds. Most commonly found at the leg region like femur, tibiotarsus, tarsometatarasal, and humerus. The surgical management and treatment was given to the fractured sites. On examination of the blood smear, no haemoprotozoan parasites could be detected but it revealed more numbers of immature round red blood cells with round to oval nucleus containing slight basophilic cytoplasm (Fig.1). This morphology of red blood cells was suggestive of anaemic changes due to asynchronous maturation of cell nucleus and the cytoplasm [2]. The matured Red blood cells of Painted storks were uniform in size, shape and color. They were typically oval or ellipitical shaped RBCS (Fig. 2) with a centrally placed oval nucleus and it contains dense and dark-staining chromatin [1].

On haematological examination, most of the birds revealed reduction in haemoglobin, packed cell volume, Mean corpuscular volume and Mean haemoglobin concentration level shown in table 1. The reduction in MCV and MCHC indicates microcytic hypochromic anaemia in the affected birds. The differential leucocyte count, on an average revealed more numbers of lymphocytes (Fig.3), followed by heterophils, monocytes and eosinophils (Fig. 4). The morphology of the erythrocytes and white blood cells were similar to that of other avian species [1, 8]. The reduction in the haematological values maybe due to the haemorrhage from the fractured sites.

In the serum biochemical analysis, the blood urea nitrogen, creatinine, alanine transaminase (ALT), aspartate transferase (AST), creatine phosphokinase (CPK), alkaline phosphatase (ALP), total protein, glucose, calcium, phosphorous, sodium, potassium, and chloride were estimated and the results were shown in table 2. The biochemical evaluation revealed marked elevation in AST, CPK andALP, which indicates there is a clear damage to the muscle and the bone due to the crush injury and fracture at different sites of the birds. The aspartate transferase is used to evaluate the activity of the liver damage or the muscle damage, but to confirm the skeletal muscle damage (e.g. trauma, feather pecking, and injections) the creatine phosphokinase has to be evaluated along with the AST [1, 7]. The increase in alkaline phosphatase in mammals, indicates the biliary diseases associated with cholestasis and bone diseases associated with increased osteoblastic activity. In avian species at this time, it indicates the elevation in ALP have been associated only with osteoblastic activity at the fractured site [4]. The values of the other parameters like blood urea nitrogen (BUN), creatinine, Alanine amino transaminase (ALT) and glucose were within the normal range similar to the other psittacine birds [6].

Fig 1: Painted Stork – Blood collection – Wing vein

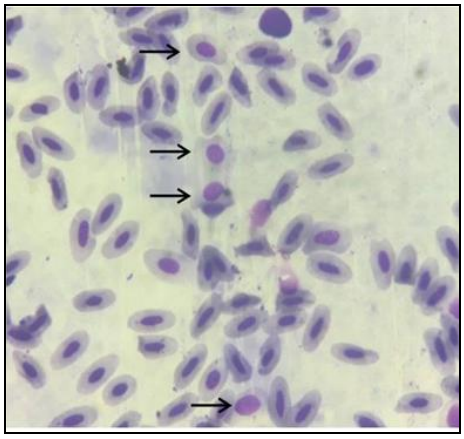

Fig 2: Painted Stork - Blood smear - Immature round red blood cells with round to oval nucleus and slight basophilic cytoplasm

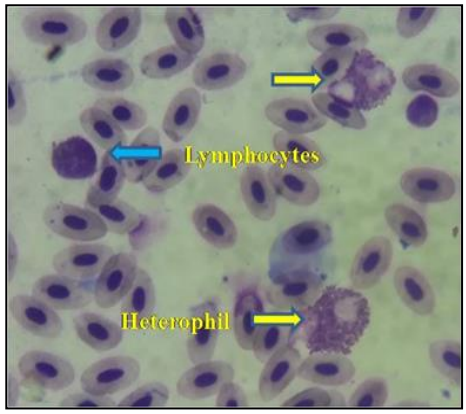

Fig 3: Painted Stork - Blood smear – Lymphocytes (blue arrow) and Heterophils (yellow arrow)

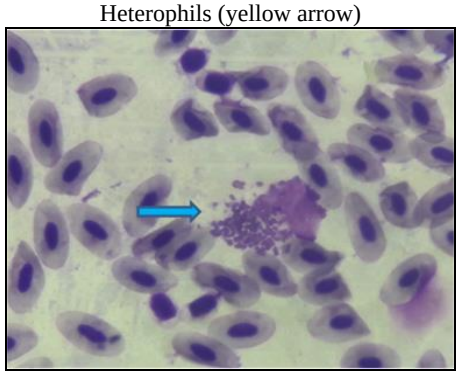

Fig 4: Painted stork – Blood smear – Eosinophil – Round to spherical red orange eosinophilic granules in cytoplasm with bilobed nucleus

Conclusion

The study of haematological and biochemical values in relation with clinical examination is the cornerstone of medical diagnosis for any species. The study of haematobiochemical alterations in various disease processes has to be initiated in water birds and other migratory birds. The results provided in this study maybe a guide for further study and related research in painted storks as there is a lacuna in haemato-biochemical alterations in painted storks.

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Conflict of interest

All the authors declare that there is no conflict of interest.

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