MICROANATOMICAL STUDY OF WHITE BLOOD CORPUSCLES OF EMU (*DROMAIUSNOVAEHOLLANDIAE*) AND ITS DIAGNOSTIC SIGNIFICANCE

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ABSTRACT

The present study was conducted to study the white blood corpuscles of Emu. The blood samples were collected from eight apparently healthy adult Emu birds and blood smears were stained with modified Wright stain for cyto-morphological studies of various white blood corpuscles. The Heterophils were round cells with lobed nucleus which was placed eccentrically. The Eosinophils often had a bi-lobed nucleus with abundant small, round, red - to - pink granules and light blue cytoplasm. The basophils cytoplasm had densely packed metachromatically stained granules. The small lymphocytes were irregularly round with round nucleus and a lacy chromatin pattern. The large lymphocytes had a homogeneous and an abundant cytoplasm which was more basophilic. The Monocytes were large cells with moderate amounts of blue - gray cytoplasm that occasionally had small discrete vacuoles. Their nuclei were pleomorphic with a lacy chromatin pattern. Deviation from the normal morphology of White Blood Corpuscles indicates disease and pathological conditions in Emu.

Key words: Emu, White Blood Corpuscles, Morphology of leucocytes

INTRODUCTION

Emu is a ratite and flightless bird, native of Australia. It is an omnivorous species with cursorial life-style and is the second largest bird in height, after the Ostrich. These birds can be well maintained on extensive (ranches) and semi intensive rearing systems with reasonably high fibrous diets. Indians are discovering the profit potential of Emus. The usefulness of ratite hematology is limited by technical difficulties similar to other avian species having nucleated erythrocytes and thrombocytes, however, their large blood volume means that ample blood can be obtained easily for evaluation. Unfortunately, wide laboratory - to - laboratory differences in reference intervals and sparse clinical information concerning hematologic changes expected with specific ratite diseases frustrate the interpretation of ratite hematology. However, once a specific disease problem is identified in a flock, knowing the status of Heterophils and hematocrit in individual birds can be quite useful in the treatment and evaluation of the disease in the flock (Douglas and Jane,2010).Considering that progress in avian hematology is based largely on a few domesticated

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species, the results of this research could be of importance as comparative data of avian living in their natural habitat and farmed birds.

MATERIALS AND METHODS

The present study was conducted in 8 normal and healthy adult Emu birds (*Dromaiusnovaehollandiae)*. The birds were maintained under optimum conditions of feed and management at a private Poultry Farm, Anand, Gujarat. The blood sample of about 2-3 ml was collected aseptically from the right external jugular vein, in the vacutainer containing K_3E.D.T.A. The blood smears were prepared directly from fresh blood immediately on a clean glass slide and stained with Modified Wright stain (300 mg of Wright stain & 30 mg of Giemsa stain grounded in mortar until dissolved in 100 ml of absolute methyl alcohol) for cyto-morphological studies of various white blood corpuscles (WBC). The staining procedure of Jain (1986) was followed for staining of the blood smears.

RESULTS AND DISCUSSION

The blood smears of Emu were stained and studied under the microscope using the oil immersion under 100X. The leukocytes studied in the blood smear of Emu are divided into granulocytes (heterophils, eosinophils and basophils) and agranulocytes (lymphocytes and monocytes).

GRANULOCYTES:

Heterophils

The heterophils of Emu were round shaped cells and were relatively larger cells (Fig.1). The background cytoplasm of Heterophil of Emu was clear as pointed by Hawkeyand Dennett (1989) in mature avian heterophils and Douglas and Jane (2010) in Ratite. The heterophil is a rounded cell as stated by Maxwell & Robertson (1998) in avian species and Bounous and Stedman (2000) in chicken and turkey. Immature heterophils are uncommon in normal ratite blood, and their stage of maturation is based on nuclear shapes, similar to other species. The cytoplasm of immature heterophils is slightly more basophilic than that of mature heterophils(Douglas and Jane, 2010). The cell is often partially occluded by numerous round to fusiform red - orange colored granules as stated by Lucas &Jamroz (1961) in domestic fowl, Sturky and Griminger (1986) in chicken and Maxwell and Robertson (1998) in avian heterophil. Randall and Reece (1996) have observed that the cytoplasmic granules of heterophils of all avian species were acidophilic in nature, which is similar to the findings of this study. The nucleus of the Heterophils was usually segmented as reported by Douglas and Jane (2010) in Ratite and isbilobulated or trilobulated as stated by Sturky&Griminger (1986) in chicken and Bounous and Stedman (2000) in chicken and Turkey. Thrall *et al.,* (2004) have reported that the nucleus of mature heterophil in healthy birds, was lobed with coarse, clumped chromatin material. Phillip *et al*., (2009) have mentioned that the Struthioniformesheterophils had prominent fusiform, brick-red to dull brown colored granules that were present at high density, which are sililar to the findings of this study. The nucleus was placed eccentrically as reported byBonadiman*et al*.,(2009) in Ostrich heterophil. The granulation of immature ratite heterophils ranges from early cells with moderate numbers of basophilic round (primary) granules, to intermediate cells having mostly round eosinophilic granules, to mature cells having mostly orange fusiform granules. Increased immature heterophils indicates inflammatory diseases of ratites. In diseases associated with toxicity, it is common to see abnormal heterophil granulation characterized by cells that are poorly granulated or retain the round primary granules of early precursors. In severe toxicity, heterophils may partially degranulate, nuclear swelling occurs, and their cytoplasm becomes more basophilic.

Inflammatory diseases of ratites associated with heterophilic left shifts and toxic changes are usually associated with a poor prognosis (Douglas and Jane, 2010).

Eosinophils

The Eosinophils of Emu is a round cell with light blue colour cytoplasm (Fig.2,3) as observed by Maxwell in 1987 in Avian eosinophil and Bounousand Stedman (2000) in Chicken and Turkey. Eosinophils of Emu had abundant small, round bright pink or eosininophilic granules as observed by Bounousand Stedman (2000) in Chicken and Turkey ,Thrall *et al.,* (2004) in Avian Eosinophil andDouglas and Jane (2010) in Ratite. Bonadiman*et al*., (2009) reported that the eosinophils of Ostrich had many eosinophilic, round granules which was homogeneously distributed, which was the similar observations in eosinophils of Emu. Eosinophils often had a bilobed nucleus and was eccentrically placed as reported by Douglass and Jane (2010) in Ratite. The nucleus was basophilic as reported by Phillip *et al*., in 2009 in Struthioniformes. The granules of ratite eosinophil precursors are larger, and primary granules may also be present in their cytoplasm. Parasitism appears to induce eosinophilia less commonly in ratites than in mammals or certain other avian species (particularly raptors)(Douglas and Jane, 2010).

Basophils

The Basophils of Emu were found to be slightly smaller than the heterophil as reported by Douglass and Jane (2010) in Ratite. They were round cells (Fig.4) as reported by Bounousand Stedman (2000) in Chicken and Turkey. The basophils had a moderate amount of cytoplasm and often had a distinct purple hue. The cytoplasm had densely packed, metachromatically stained granules as stated by Deldar (1998), Thrall *et al.,* (2004) in Avian Basophils, Phillip *et al*., (2009) in Struthioniformes and Douglass and Jane (2010) in Ratite. The granules were larger in congruence with the reports of Bonadiman*et al*., (2009) in Ostrich and were bigger than the granules of eosinophil and overlying each other. Although basophil function is unclear in ratites, basophilia is occasionally noted in hypersensitivity reactions and chronic respiratory diseases (Douglas and Jane, 2010).

AGRANULOCYTES:

Lymphocytes

Lymphocytes of Emu varied in size from small lymphocytes to large lymphocytes as stated by Deldar in Aves in 1998. The small lymphocytes of Emu (Fig.5) were irregularly round cells as reported by Bounousand Stedman (2000) in Chicken and Turkey and Thrall *et al*., (2004) in Avian lymphocytes. The nuclear shape of small lymphocytes was round as reported by Deldar (1998) in Avian lymphocytes and had a lacy chromatin pattern. Small lymphocyte had high nucleus: cytoplasm ratio. Their cytoplasm varied in amount from scant to moderate and was usually lightly basophilic. In Ratite this feature is quite helpful in distinguishing small lymphocytes from activated thrombocytes having clear cytoplasm. Degenerate lymphocytes may have scant cytoplasm or cytoplasmic blebs (Douglas and Jane, 2010).

The large lymphocytes of Emu (Fig.3,7) had comparatively less dense nucleus, which was eccentrically placed. Deldar (1998) studied the avian lymphocytes and found that the nucleus of some large lymphocyte showed nuclear indentations, which is similar to the findings of study. The large lymphocytes had a homogeneous and an abundant cytoplasm which was more basophilic. Generally the cytoplasm of small and large lymphocytes lacked both vacuoles and granules (Fig.3,5,7) as described

by Thrall *et al*., (2004) in Avian lymphocytes. Plasmacytoid or reactive lymphocytes may be seen in ratites, particularly during convalescent immune responses .These plasma cell - like lymphocytes have an eccentric round nucleus, dark basophilic cytoplasm, and a pale perinuclear Golgi zone. Mild lymphocytosis is also associated with immune stimulation, noted particularly during convalescence. Marked lymphocytosis of either small or larger lymphoid cells is suggestive of lymphocytic leukemia in ratites (Douglas and Jane, 2010).

Monocytes

Monocytes of Emu were large cells with moderate amounts of blue - gray cytoplasm that occasionally had small discrete vacuoles (Fig.6,7) as reported by Deldar (1998), Thrall *et al*., (2004) in avian Monocytes and Douglas and Jane (2010) in Ratite. Their nuclei were pleomorphic with a chromatin pattern that is lacy and somewhat less condensed than that in the lymphocytes which is similar to the findings of Bonadiman*et al*., (2009) in Ostrich. Distinct cytoplasmic granules were uncommon in monocytes, although faint, dust - like eosinophilic granules were noted in few cells as reported by Thrall *et al*., (2004) in avian Monocytes, Bonadiman*et al*., (2009) in Ostrich Monocyte and Douglas and Jane (2010) in Ratite. Differentiating monocytes from enlarged reactive lymphocytes can be challenging in ratite blood smears. Monocytosis is associated with various chronic granulomatous diseases of ratites, including mycoses, and diseases in which marked tissue necrosis occurs (Douglas and Jane, 2010).

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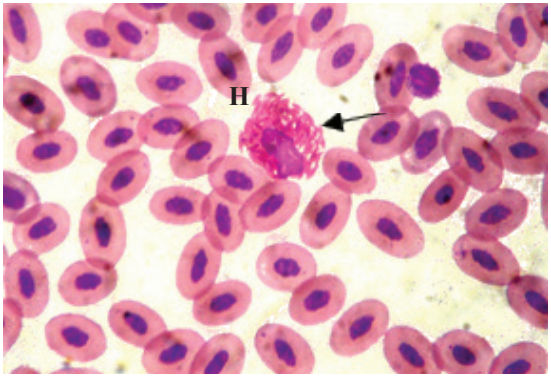

Fig.1: Blood smear showing the Heterophil (H) with fusiform shaped pinkish granules (arrow) and colorless cytoplasm. (Modified Wright's Stain X 100)

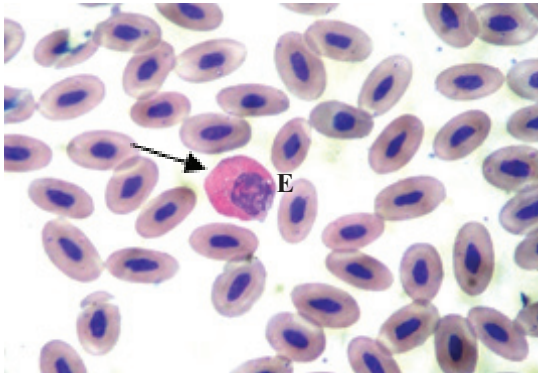

Fig.2: Blood smear showing the Eosinophil (E) with spherical pinkish granules and a pale blue cytoplasm (arrow). (Modified Wright's Stain, X 100)

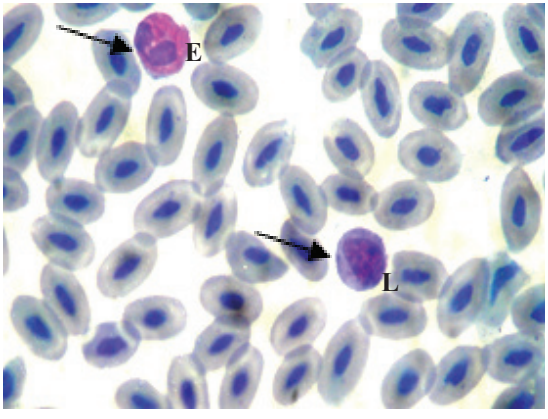

Fig.3: Blood smear showing the Eosinophil (E) with bi-lobed nucleus connected by chromatin strand (arrow). A large lymphocyte (L) with basophilic cytoplasm can also be observed. (Modified Wright's Stain, X 100)

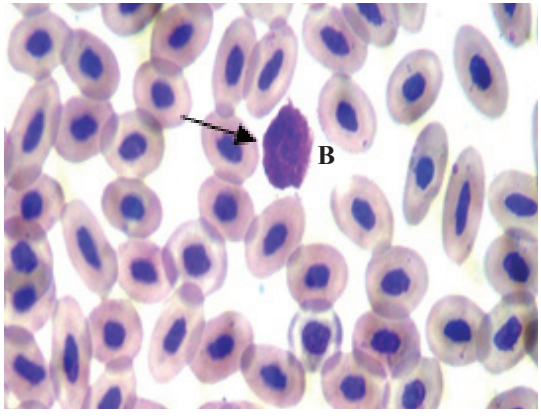

Fig.4: Blood smear showing the Basophil (B) with large sized round, bluish abundant granules masking the nucleus. (Modified Wright's Stain, X 100)

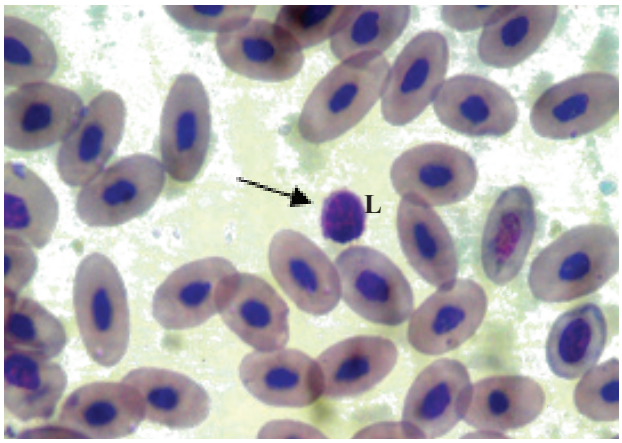

Fig.5: Blood smear showing the small lymphocyte (L) with small sized bluish spherical nucleus and a rim of scanty cytoplasm. (Modified Wright's Stain, X 100)

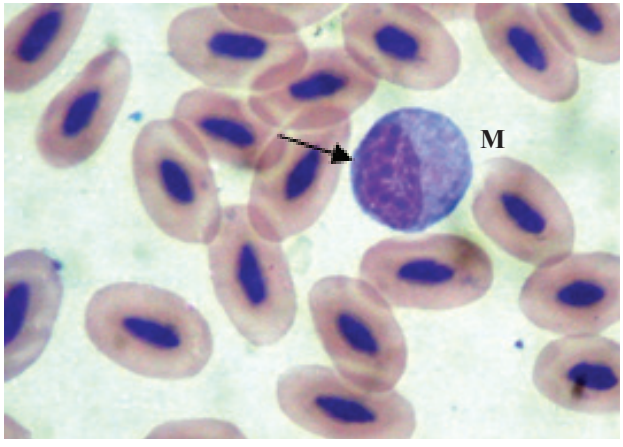

Fig.6: Blood smear showing the Monocytes (M) with abundant cytoplasm with small vacuoles and faint, dust - like eosinophilic granules (arrow) and the nuclei with lacy chromatin pattern.
(Modified Wright's Stain, X100)

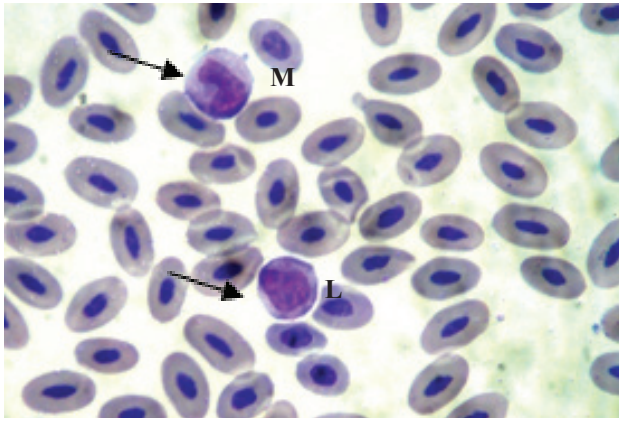

Fig.7: Blood smear showing the large sized lymphocyte (L) with bluish spherical nucleus and a monocyte (M) with indented nucleus.
(Modified Wright's Stain, X 100)