

Research Article

Cytology of Bone Marrow Haematopoietic Cells of Village Weavers (*Ploceus cucullatus*)

¹A.T. Adetuga, ²A.J. Jubril, ¹A.O. Omonona and ²T. Omadevuaye

¹Department of Wildlife and Ecotourism Management, University of Ibadan, Ibadan, Nigeria

²Department of Veterinary Pathology, University of Ibadan, Ibadan, Nigeria

Abstract: In order to study the cellular morphology and Myeloid/Erythroid (M/E) ratio in the bone marrow of the adult village weaver birds (*Ploceus cucullatus*), bone marrow samples were collected from the proximal tibiotarsus bone of 30 clinically-healthy adult village weaver birds during post mortem. The bone marrow smears were fixed and stained using the Romanowsky Giemsa Stain (RGS), then viewed microscopically. Blood samples were also collected ante mortem for routine haematological examination. The results showed that the mean M/E ratio was 0.19, the mean erythroid cell percentage was 72.93, the mean myeloid cell percentage was 19.8 and the mean percentage of all other cells was 0.33. The Myeloid/Erythroid (M/E) ratio obtained from this study is comparable to that of quails. The morphology and distribution of blood cells in the bone marrow of adult village weaver birds were similar with reports from other birds such as ducks and black-head gull. Other findings from this study are comparable to existing data in the literature. The peripheral blood evaluation was within reference ranges. There is need to further determine the mean sizes of all cells of the bone marrow of village weaver birds and compare with that of other birds.

Keywords: Avian, bone marrow, cell morphology, cytology, myeloid/erythroid ratio, village weaver

INTRODUCTION

Haematopoiesis occurs primarily in the bone marrow of post-hatch birds, however, haematopoietic activity may also be found in various internal organs like liver and possibly spleen (Kim, 2010; Lopez *et al.*, 2014). The most appropriate evaluation of the haematopoietic system involves careful evaluation of the peripheral blood haemogram and bone marrow examination (Lee *et al.*, 2008; Tadjalli *et al.*, 2012). Bone marrow is the soft, spongy, gelatinous tissue found in the hollow spaces in the interior of bones (Rubin and Strayer, 2008). It is either red marrow, containing haemopoietic cells, or yellow marrow, which is largely adipose tissue (Bain *et al.*, 2010). The bone marrow is the definitive hematopoietic tissue containing the pluripotent hematopoietic stem cells that differentiates along all the blood cell lineages. It is found within the deepest depths of bones filling the space between the bone and the trabecular beams (Dean, 2005; Kulikov *et al.*, 2014). It performs integral functions in the body of living organisms, which include but not limited to haematopoiesis; partakes in body metabolic processes; ensuring bone strength, elasticity and hardness (Kulikov *et al.*, 2016; Taichman, 2005). Bone marrow consists of a hematopoietic cell compartment and a stromal component that supports

proliferation of the haematopoietic cells (Tripathy and Dudani, 2013). The localization of the bone marrow in various parts of the skeleton of vertebrates varies considerably in different genera of animals. The bone marrow in birds is localized mainly in the peripheral skeleton and about 45% in the humeral, femoral and tibia bones, whereas its quantity is very small in the axial skeleton or is sometimes not present (Almany Magal *et al.*, 2014). This is contrary to what is obtainable in mammals where the main mass of the bone marrow is found in the axial skeleton (Farhi, 2009).

Bone Marrow Examination (BME) can provide important information and is considered a valuable diagnostic tool to evaluate various haematological disorders (Chand *et al.*, 2015). The expediency of bone marrow aspiration as a diagnostic tool depends on appropriate collection and handling of the sample and a knowledge of normal marrow morphology (Harvey, 1984; Kaur *et al.*, 2014). For proper comparative evaluation, it is pertinent that blood samples are collected together with the bone marrow specimen (Weiss and Wardrop, 2010). Indicators of marrow examination in avian species are similar to mammals and have been documented (Samour, 2000). Bone marrow examination has been indicated for certain abnormal haematological findings such as unexplained

cytopenias (any non-regenerative anaemia), maturation defects, morphologic abnormalities in blood cells, suspected myeloproliferative diseases, malignancies metastatic to marrow and other unexplained cellular changes in the peripheral blood (Campbell, 1988; Chand *et al.*, 2015). The morphology of the bone marrow and its examination is best derived from a properly prepared direct smear because the cells are less manipulated and no anticoagulant is used with this technique. Variables that can affect the quality of the smear include the size of the aspirate marrow drop, the angle and the speed of spreading the smear and the haematocrit of the sample. As such, selection of the best quality direct smear is critical for accurate interpretation. Bone marrow sampling could be done either through bone marrow aspiration or bone marrow core biopsy. Bone marrow smears/aspirates are the best samples for evaluating cellular morphology and also the ratio of white cell lineage to red cell lineage (myeloid : erythroid or M:E ratio), which gives a rough estimate of where the marrow's replicative energies are directed. The fine structure and hematopoietic cell morphology of bone marrow of birds such as ducks, Japanese quails and black head gull have been investigated and documented (Tadjalli *et al.*, 1997; Nazifi *et al.*, 1999; Tadjalli *et al.*, 2002). However there is little or no known information available on the hematopoietic cells of the village weaver bird. This study therefore aims to determine the bone marrow cell morphology and myeloid/erythroid ratio in village weaver birds within Ibadan Metropolis, Nigeria.

METHODOLOGY

Bone marrow aspirates were taken from 30 clinically-healthy adult village weaver birds with an average weight of 32 g captured from roosting sites within Ibadan Metropolis, Nigeria. They were captured at night with net traps and allowed to stabilize for two weeks prior to collection of samples. Bone marrow aspirates were collected from the proximal tibiotarsus bone of the bird's post-mortem. For each village weaver bird, several bone smears were made on pre-cleaned microscope slides, fixed in 99% methanol and stained with RGS. Stained slides were thereafter evaluated for cellularity, cell morphology and classification of erythroid, myeloid and thrombocytic precursors. Sequel to this, each sample was used for a 500-cell differential count to classify the bone marrow precursors in each cell series and to evaluate the Myeloid: Erythroid (M/E) ratios for each village weaver bird. Specifically, the M/E ratio was determined by dividing the total of all the nucleated cells of the granulocytic series by the total of all the nucleated cells of the erythrocytic series following the method of Jain (1993). The classification of the myeloid series included myeloblasts, promyelocytes, myelocytes, metamyelocytes, bands and segmenters while the

Table 1: Cellular composition of the bone marrow of adult village weaver birds

Erythroid series	Cells (%)
Rubriblast	3.23±2.54
Prorubricyte	4.90±4.88
Basophilic rubricyte	7.13±6.23
Early polychromatophilic rubricyte	26.20±15.13
Late polychromatophilic rubricyte	31.47±20.24
Total erythroid cells	72.93±12.02
Myeloid series	
Myeloblast	5.00±4.92
Promyelocyte	6.50±5.22
Myelocyte, heterophilic	2.17±2.36
Myelocyte, eosinophilic	0.50±1.07
Myelocyte, basophilic	1.10±2.82
Metamyelocyte, heterophilic	1.50±1.68
Metamyelocyte, eosinophilic	1.43±2.56
Metamyelocyte, basophilic	0.77±1.55
Band cell	0.83±1.18
Total myeloid cells	19.80±2.59
Thromboblast	0.87±2.45
Thrombocyte	0.17±0.46
Total thrombocytic cells	1.04±1.46
Mitotic cells	0.30±0.84
Osteoclast	0.03±0.18
Plasma cell	0.00±0.00
Total other cells	0.33±0.34
Myeloid: Erythroid ratio (M/E)	0.19±0.09

Percentages are given as Mean±S.D.

classification of the erythroid series included rubriblasts, prorubricytes, basophilic rubricytes, early polychromatophilic rubricytes and late polychromatophilic rubricytes. Blood samples were also collected ante mortem for routine haematological examination. The blood profile of the birds has been evaluated to be in the range of normal value. The results have been used in another publication (Omonona *et al.*, 2014). Average statistical mean±standard deviation was used for the description of the result using Statistical Package (SPSS version 20). Statistical significance was set at $\alpha_{0.05}$.

RESULTS

The cellular composition of the bone marrow of village weaver birds showed that the mean percentage for erythroid and myeloid cells were 72.93 and 19.8 respectively. The findings from this study also revealed that the highest percentage of cells were late polychromatophilic rubricyte in the erythroid series and promyelocytes in the myeloid series as shown in Table 1. The mean M/E ratio was 0.19.

The Photomicrograph of hematopoietic cellular composition of the bone marrow of adult village weavers is shown in Fig. 1 to 4. Different cell in the cell lines and distribution were observed and shown.

Rubriblasts were the biggest in the erythroid series, with large central round nuclei, making the nucleus-cytoplasm ratio high, a nucleoli and deeply basophilic vacuolated cytoplasm. The Prorubricytes is similar in morphology to the rubriblasts, but the chromatin was

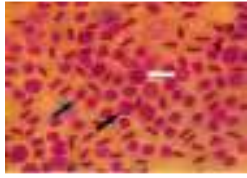


Fig 1: Photomicrograph of haematopoietic cells in bone marrow of adult village weavers showing high population of erythroid cells, basophilic rubricyte (white arrow), early polychromatophilic rubricyte (black arrow) and late polychromatophilic rubricyte (blue arrow) x1000 magnification

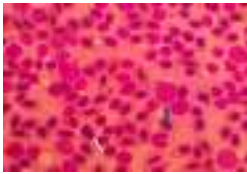


Fig. 2: Photomicrograph of haematopoietic cells in bone marrow of adult village weavers showing high population of erythroid cells, a myeloblast (blue arrow) and a mitotic figure (white arrow) x1000 magnification



Fig. 3: Photomicrograph of haematopoietic cells in bone marrow of adult village weavers showing a myelocyte (blue arrow) and a band heterophil (white arrow) x1000

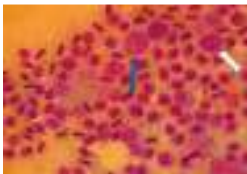


Fig. 4: Photomicrograph of haematopoietic cells of the bone marrow of adult village weavers with high population of erythroid cells, a rubriblast (blue arrow) and a prorubricyte (white arrow) x1000

denser, the nucleoli were less distinct. Basophilic rubricytes are similar to prorubricytes, but with a round nucleus containing clumped chromatin (Fig. 4).

Early polychromatophilic rubricytes and late polychromatophilic rubricytes are similar but have distinct features such as sharp, colour and features. The early polychromatophilic rubricytes is round while the late polychromatophilic rubricytes is oval in shape. The nuclei of both cells are small compared to other cells reducing the nuclei/cytoplasmic ratio. The nuclei of the

former has even clumped chromatin while the latter has irregularly clumped chromatin (Fig. 1).

Myeloid series cells are larger, with pale cytoplasm. The myeloblasts were the largest cells in the series, are round with a round nuclei and a narrow rim of blue cytoplasm (Fig. 2). The nuclei chromatin is reticular with prominent nucleoli. The promyelocytes are similar to myelocytes but the nuclei were eccentric and the cytoplasm contained dark granules. Myelocytes had reduced in size compared to the promyelocytes and were smaller and spherical compared to the promyelocytes (Fig. 3). The cytoplasm contained secondary specific granules which classify each as heterophilic, eosinophilic, or basophilic. The metamyelocytes of heterophil, eosinophil and basophil were smaller than the myelocyte. The nuclei were slightly indented and the cytoplasm had more obvious specific granules. The Band cells are similar to the metamyelocyte but with a lightly elongated nuclei (Fig. 3). The mature granulocyte has the lobed nucleus. The routine haematological examinations were within the reference range as had been reported in a previous article (Omonona *et al.*, 2014).

DISCUSSION

One of the keys to understanding, disclosure and prevention of pathology in the haematopoietic tissue is the knowledge of the structural organization and patterns of development of the bone marrow (Kulikov *et al.*, 2016). As such, there is need for bone marrow examination and evaluation, which is critical to assessing haematopoietic function and investigating the aetiology of abnormal peripheral blood counts. Also, definitive diagnosis of several haematological diseases and other bone marrow disorders has been reported to mostly require bone marrow examination (Moix *et al.*, 2008). According to Chand *et al.* (2015), a thorough bone marrow morphological examination includes Peripheral Blood Film (PBF), direct particle, buffy coat, bone marrow aspiration smears, trephine biopsy imprints for sections and marrow volumetric data respectively. Microscopic evaluation is often performed on cytology samples (bone marrow smears/aspirates) and on histology samples (core biopsies). There are several indications for performing a bone marrow examination especially in avian species. These include further workup of haematological abnormalities observed in the peripheral blood smear, evaluation of primary bone marrow tumours, staging for bone marrow involvement by metastatic tumours, assessment of infectious disease processes including fever of unknown origin and evaluation of metabolic storage diseases (Green *et al.*, 2003). In this study, the bone marrow examination in the species is important for baseline data for the species, which is rare in literature. The species is becoming a good sentinel for eco-toxicological studies, therefore a need for

documentation of the baseline parameters, including bone marrow cytology. From this study, the mean value for the Myeloid/Erythroid (M/E) ratio which was estimated in the context of the overall cellularity (an estimate of the percentage of haematopoietic components and stroma in the marrow space) was 0.19. This value is comparable with that of quail (Nazifi *et al.*, 1999) and goat (Weiss and Wardrop, 2010). The ratio is also far < the 1.24, which was reported for pheasants (*Phasianus colchicus*) by Tadjalli *et al.* (2013). The findings from this study further revealed that the highest and lowest percentages of cells in the erythroid series were late polychromatophilic rubricytes and rubriblasts respectively. These findings were similar to that of Tadjalli *et al.* (2013) in pheasants. Furthermore, in the myeloid series, the highest percentages of cells were the promyelocytes. In the present study, monoblasts (which have not been seen in the bone marrow of birds) were also not obviously observed during the bone marrow examination of the sampled village weaver birds. This is in consonance with the findings of Nazifi *et al.* (1999), Bounous and Stedman (2000) and Tadjalli *et al.* (2002). The incidence of thromboplast and thrombocyte mean percentages observed in this study were very low. Avian thrombocytes are often derived from mononuclear precursor cells unlike mammalian platelets that are cytoplasmic fragments of megakaryocytes (Bounous and Stedman, 2000). The mitotic cells, plasma cells and osteoclasts were also seen in very low percentages, are similar to other reported avian species and are in contrast, comparable to other domestic species (Weiss and Wardrop, 2010). The routine haematological examinations that were within the reference range indicate that the birds were relatively healthy and the bone marrow data can represent healthy birds.

CONCLUSION

Bone marrow cellularity of village weaver birds (*Ploceus cucullatus*) captured within Ibadan Metropolis was assessed from aspirated bone marrow fragments in wedge-spread film. The Myeloid/Erythroid (M/E) ratio obtained from this study was comparable to that of quails. Other findings from this study are comparable to existing data in the literature.

RECOMMENDATION

There is need to further determine the mean sizes of the erythroid, myeloid and other cellular series of the bone marrow of village weaver birds and compare with that of other birds.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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