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Evaluation of Haematopoietic Cells and M/E Ratio in the Bone Marrow of the Partridge (*Alectoris chukar*)

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Abstract: In order to study the normal haematopoiesis, cellular components and M/E ratio in the bone marrow of the partridge (*Alectoris chukar*), bone marrow samples were collected from the proximal tibiotarsus bone of 16 clinically healthy adult partridge (9 male and 7 female). The bone marrow smears were stained using the Giemsa stain. The results indicated that the development and formation of blood cells in the bone marrow of partridge were similar to other birds, whereas the morphology of the cells was similar to chickens, ducks, quail and black-head gull. The mean Myeloid/Erythroid (M/E) ratio was 1.33, the mean erythroid percentage was 39.15%, the mean myeloid percentage was 52.34% and the mean percentage of all other cells percentage was 7.45 %. There was no significant difference in any of the cellular composition between male and female.

Key words: Bone marrow, haematopoietic cells morphology, M/E ratio, partridge

INTRODUCTION

Bone marrow examination can provide valuable information about haematopoietic status (Weiss and Wardrop, 2010). The usefulness of bone marrow aspiration as a diagnostic tool depends on proper collection and handling of the sample and on a knowledge of normal bone marrow morphology (Harvey, 1984). It is imperative that a blood sample is always collected together with the bone marrow specimen for proper comparative evaluation (Weiss and Wardrop, 2010). Various indicators of marrow examination in avian patients include: nonregenerative anaemias, thrombocytopenias, heteropenias, pancytopenias, suspected leukaemia and other unexplained cellular changes in the peripheral blood Campbell (1988). Fine structure and haematopoietic cell morphology of bone marrow of chickens, pigeon, ducks, Japanese quails and black head gull have been investigated by Campbell (1967, 1988), Campbell and Coles (1986), Tadjalli *et al.* (1997), Nazifi *et al.* (1999) and Tadjalli *et al.* (2002). Haematology and blood chemistry of gulls were reported by Averbeck (1992) and Work (1996), but there is no information about the haematopoietic cells of the partridge. The purpose of the present study, therefore, was to determine the bone marrow cell morphology and M/E ratio in partridge.

MATERIALS AND METHODS

Bone marrow aspirations were obtained from 16 clinically healthy adult partridge (9 male and 7 female). All birds were free of haematological abnormalities on peripheral blood examination. The medial aspect of the proximal tibiotarsus bone, just below the femoral-tibiotarsal joint, was aseptically prepared and 22 gauge disposable marrow aspiration needles were used to obtain samples. The area was anaesthetized locally by subcutaneous infiltration of 1-1.5 ml of 2% lignocaine HCl over the periosteum. The aspiration biopsy needle was held perpendicular to the bone and advanced in to the marrow space by applying light pressure and using slight rotatory motions. With the needle in the marrow space, the stylet was removed and a syring was locked into the needle. The samples were collected into 5 ml syringes containing EDTA. At least five air-dried wedge slides of bone marrow smears were prepared from each partridge. Slides were stained with Giemsa and were evaluated for cellularity and classification of erythroid, myeloid and thrombocytic precursors. Each sample was used for a 500-cell differential count to classify the marrow precursors in each cell series and to determine Myeloid:Erythroid (M/E) ratios for each partridge.

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 (Jain, 1986). The classification of the erythroid series included rubriblasts, prorubricytes, basophilic rubricytes, early polychromatophilic rubricytes and late polychromatophilic rubricytes. The classification of the myeloid series included myeloblasts, promyelocytes, metamyelocytes, bands and segmenteds. The results were expressed as means ±SEM. All data were done with the Statistical Package for Social Sciences (SPSS 16.0 for windows). The results were analyzed using T test for comparison between two sexes. Statistical significance was set at $p<0.05$.

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Table 1: Cellular composition of the bone marrow of male (n = 9) and female (n = 7) partridge

Cells	F (%)	M (%)	Cells	F (%)	M (%)	Cells	F (%)	M (%)
Rubriblast	0.71±0.18	2.33±0.88	Myeloblast	0.71±0.40	0.44±0.15	Mitotic cells	1.00±0.21	0.66±0.23
Prorubricyte	3.14±014	4.39±0.56	Promyelocyte	3.86±0.40	4.17±0.56	Osteoclast	0.57±0.29	0.61±0.26
Basophilic rubricyte	5.14±0.34	3.72±0.49	Myelocyte, basophilic	1.86±0.26	2.83±0.56	Plasma cell	0.85±0.26	0.88±0.26
Early polychromatophilic rubricyte	23.85±0.40	23.50±0.98	Myelocyte, heterophilic	23.14±0.59	23.44±0.69	Thrombocyte	2.85±0.34	1.94±0.31
Late polychromatophilic rubricyte	6.42±0.36	5.11±1.05	Metamyelocyte	14.29±0.28	13.67±1.19	Monocyte	0.42±0.29	1.16±0.40
Total erythroid cells	39.26±0.29	39.05±0.79	Band	5.57±0.29	7.11±0.85	Lymphocyte	1.14±0.34	1.72±0.71
			Heterophil	1.14±0.34	0.94±0.29	Degenerate cell	0.43±0.20	0.67±0.23
			Eosinophil	0.71±0.18	0.55±0.17	Total other cells	7.26±0.27	7.64±0.34
			Basophil	0.14±0.14	0.11±0.11			
			Total myeloid cells	51.42±0.32	53.26±0.51	Myeloid:Erythroid ratio (M/E)	1.33±0.38	

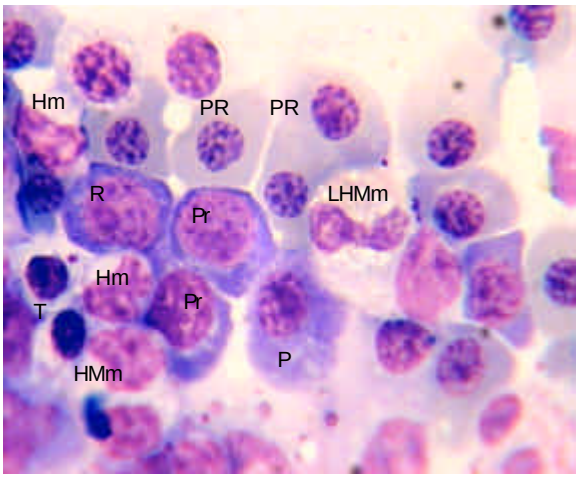

Fig. 1: Photomicrograph of haematopoietic cells in female partridge (Giemsa x 1800). R: Rubriblast; Pr: Prorubricyte; PR: Early polychromatophilic rubricyte; P: Plasma cell; LHMm: Late heterophilic metamyelocyte; Hm: Heterophilic myelocyte; HMm: Heterophilic metamyelocyte; T: Thrombocyte

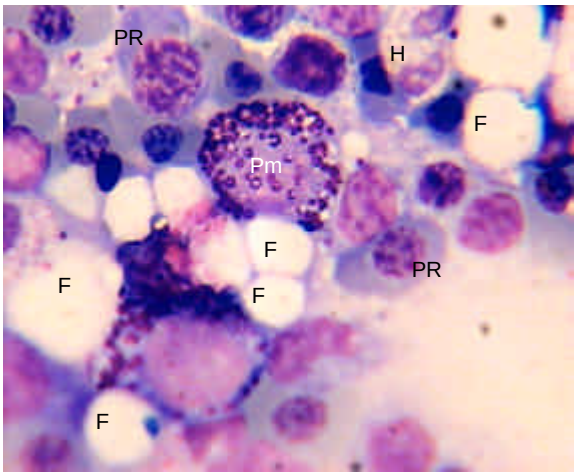

Fig. 2: Photomicrograph of haematopoietic cells in female partridge (Giemsa x 1800). PR: Early polychromatophilic rubricyte; Pm: Promyelocyte; H: Heterophil; F: Fat cells

RESULTS

The cellular composition of the bone marrow of partridge is presented in Table 1. The mean value for the M/E ratio was 1.33.

The mean percentage for erythroid and myeloid cells were 39.15% and 52.34% respectively. The finding of this study revealed that the highest percentage of cells were early polychromatophilic rubricytes in the erythroid series and myelocytes in the myeloid series. Rubriblasts were big cells with large central round nuclei with nucleoli. The N:C ratio was high. The cytoplasm was deeply basophilic and vacuolated (Fig. 1, 3, 5). Prorubricytes resembled rubriblasts, but their chromatin was more dense, nucleoli were indistinct and their cytoplasm very deeply basophilic (Fig. 1, 3, 4, 5). Basophilic rubricytes were smaller than prorubricytes with a round nucleus containing clumped chromatin (Fig. 3, 4, 5). Early polychromatophilic rubricytes were round cells with a grey (basophilic to slightly eosinophilic) cytoplasm.

The nucleus of these cells was small in relation to the cytoplasm and had clumped chromatin (Fig. 1-6). Late polychromatophilic rubricytes were approximately oval in shape with a nucleus round to slightly oval containing irregularly clumped chromatin (Fig. 3, 4, 5, 6).

Myeloblasts were large and round with a narrow rim of blue cytoplasm. Their nucleus was round with a reticular chromatin and prominent nucleoli. Promyelocytes were large round cells with light blue cytoplasm and eccentric round nucleus. Their cytoplasm contained dark magenta granules (Fig. 2). Myelocytes were smaller than promyelocytes. They had a spherical shape with an eccentric oval nucleus. Their cytoplasm contained

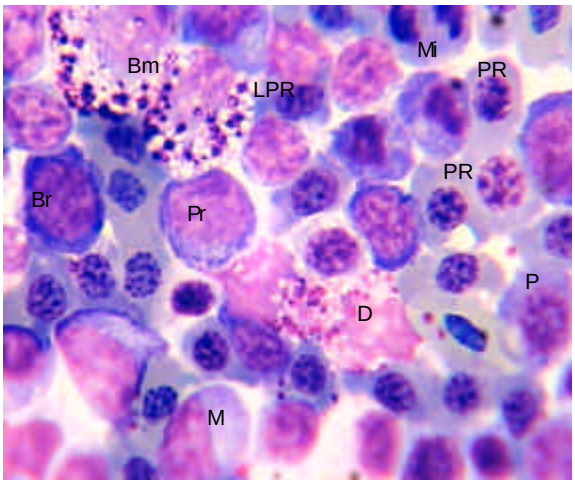

Fig. 3: Photomicrograph of haematopoietic cells in female partridge (Giemsa x 1800). LPR: Late polychromatophilic rubricyte; PR: Early polychromatophilic rubricyte; R: Rubriblast; Pr: Prorubricyte; Br: Basophilic rubricyte; M: Myelocyte; Bm: Basophilic myelocyte; P: Plasma cell; Mi: Mitotic cell; D: Degenerate cell

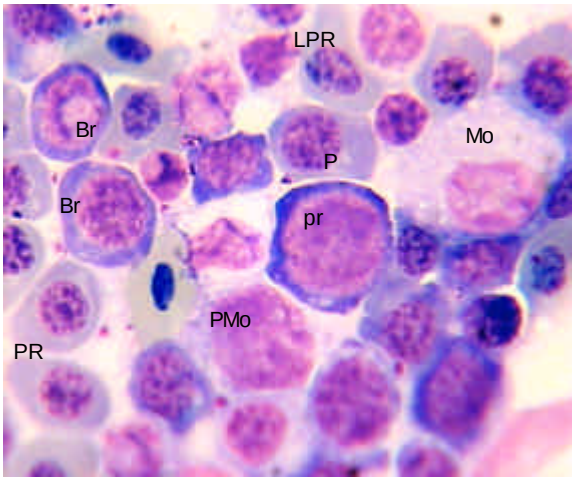

Fig. 4: Photomicrograph of haematopoietic cells in male partridge (Giemsa x 1800). LPR: Late polychromatophilic rubricyte; PR: Early polychromatophilic rubricyte; Pr: Prorubricyte; Br: Basophilic rubricyte; Mo: Monocyte; PMo: Promonocyte; P: Plasma cell

secondary granules (specific granules) which could be classified as either the heterophil, eosinophil or basophilic series. Heterophilic, eosinophilic and basophilic myelocytes contained less than half the definitive number of mature granules. Eosinophilic myelocytes lacked the magenta granules (Fig. 1, 3, 5, 6). Metamyelocytes were smaller than their precursor cells.

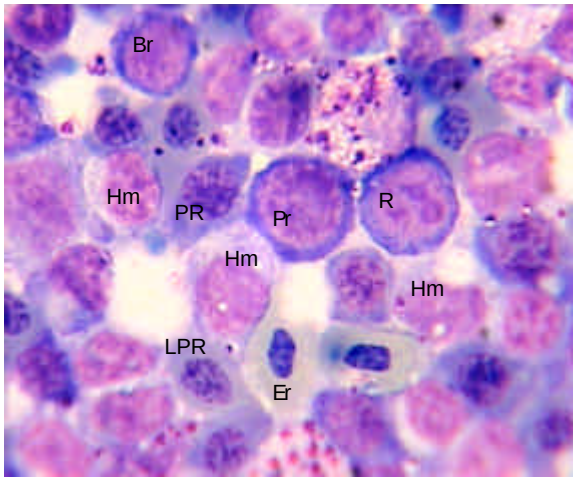

Fig. 5: Photomicrograph of haematopoietic cells in male partridge (Giemsa x 1800). LPR: Late polychromatophilic rubricyte; PR: Early polychromatophilic rubricyte; Pr: Prorubricyte; Br: Basophilic rubricyte; R: Rubriblast; Er: Erythrocyte; Hm: Heterophilic myelocyte

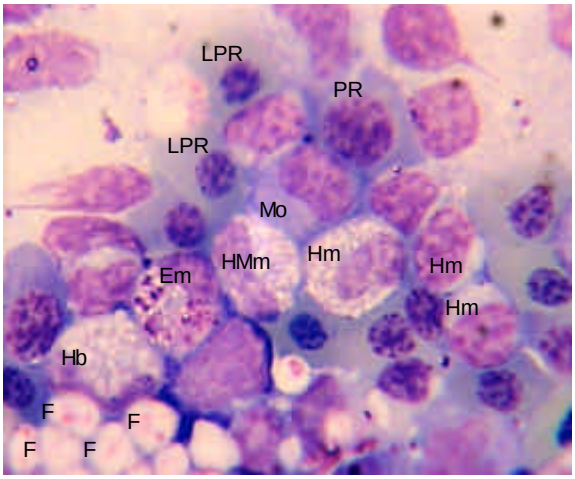

Fig. 6: Photomicrograph of haematopoietic cells in male partridge (Giemsa x 1800). PR: Early polychromatophilic rubricyte; LPR: Late polychromatophilic rubricyte; Hm: Heterophilic myelocyte; Em: Eosinophilic myelocyte; Hb: Heterophilic band; HMm: Heterophilic metamyelocyte; Mo: Monocyte; F: Fat cells

Their nucleus was slightly indented or bean shape and their cytoplasm had more than half the definitive number of specific granules (Fig. 1, 6). Band cells resembled the mature granulocyte but lacked the lobed nucleus (Fig. 6). Thromboblasts and prothrombocytes were not observed in partridge bone marrow.

Promonocytes were large cells with clear blue cytoplasm containing granules and round nuclei with a

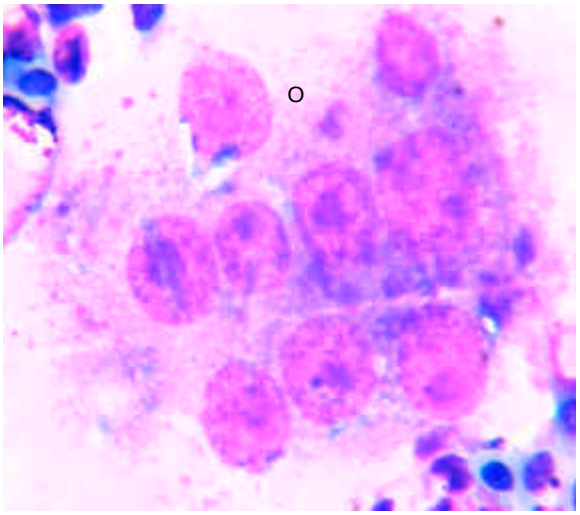

Fig. 7: Photomicrograph of haematopoietic cells in male partridge (Giemsa x 1800). O: Osteoclast

reticular nuclear chromatin (Fig. 4). The lymphoblast and prolymphocyte were not observed in partridge bone marrow samples. Plasma cells were round to oval cells with a round, eccentrically placed nucleus. A pale area of cytoplasm was observed near one side of the nucleus (Fig. 1, 3, 4). Osteoclasts were large multinucleated giant amoeboid cells. Their cytoplasm consisted of eosinophilic granules in different shapes and size and was also vacuolated. Their nuclei were round to oval with finely granular chromatin and prominent nucleoli (Fig. 7).

There was no significant difference in any of the cellular composition between male and female.

DISCUSSION

A unique feature of avian species is that erythropoiesis and possibly thrombopoiesis occur within the vascular sinuses, but granulopoiesis take place outside the vascular sinuses (Bounous and Stedman, 2000). The development and formation of blood cells in the bone marrow of partridge were similar to other birds (Campbell and Coles, 1986; Campbell, 1988), whereas the morphology of the cells was similar to chickens, pigeons, ducks, quails and black head gull (Campbell, 1967; Tadjalli *et al*., 1997; Nazifi *et al*., 1999; Tadjalli *et al*., 2002). The rubriblasts in gull bone marrows were similar to haemocytoblasts in chickens, pigeons, ducks, quails and black head gull (Campbell, 1967; Campbell and Coles, 1986; Tadjalli *et al*., 1997; Nazifi *et al*., 1999; Tadjalli *et al*., 2002). Campbell and Coles (1986) indicated that the stem cell for erythrocytes was the rubriblast (erythroblast). In the bone marrow of partridge, prorubricytes resembled rubriblasts, but the nucleolus was indistinct or absent. These prorubricytes were similar to those in chickens, ducks, quails and black head gull (Campbell and Coles, 1986; Tadjalli *et al*., 1997; Nazifi *et al*., 1999; Tadjalli *et al*., 2002). In the bone marrow of partridge, basophilic rubricytes, early polychromatophilic rubricytes and late polychromatophilic rubricytes were similar to the respective cells in chicken, pigeon, ducks, adult quail and black head gull (Campbell, 1967; Tadjalli *et al*., 1997; Nazifi *et al*., 1999; Tadjalli *et al*., 2002). Comparatively, cellular elements of erythropoiesis in birds are similar to those of mammals (Campbell and Coles, 1986; Campbell, 1988; Weiss and Wardrop, 2010). The findings of this study revealed that the highest percentages of cells were early polychromatophilic rubricytes and the lowest percentage were rubriblasts in the erythroid series. These findings were similar to those of Campbell and Coles (1986) in chickens, Tadjalli *et al*. (1997) in ducks, Nazifi *et al*. (1999) in quails and Tadjalli *et al*. (2002) in black head gull.

The granulocytic series in partridge was similar to those of other birds and mammals (Campbell and Coles, 1986; Weiss and Wardrop, 2010) and in particular to chickens (Campbell, 1967; Campbell and Coles, 1986; Campbell, 1988), ducks (Tadjalli *et al*., 1997), quails (Nazifi *et al*., 1999) and black head gull (Tadjalli *et al*., 2002). Campbell and Coles (1986) and Campbell (1988) believed that promyelocytes and progranulocytes were identical. In the present study, the highest percentages of cells in the myeloid series were related to myelocytes a finding similar to that of Tadjalli *et al*. (1997) in ducks.

Similar to ducks (Tadjalli *et al*., 1997), quails (Nazifi *et al*., 1999) and black head gull (Tadjalli *et al*., 2002) monoblasts were not observed in the bone marrow of partridge, but promonocytes were seen in low percentages. Indeed, monoblasts have not been recognized in avian bone marrow (Bounous and Stedman, 2000). It is likely that precursors of monocytes and heterophils are similar in the early stages, as in mammals and can not be distinguished by light microscopy (Campbell, 1988; Bounous and Stedman, 2000). Lymphocytes were observed in bone marrow of partridge similar to those in quails (Nazifi *et al*., 1999) and black head gull (Tadjalli *et al*., 2002). Campbell (1967) reported that bone marrow of adult chickens and pigeons contained numerous accumulations of lymphatic tissues. By contrast, Bounous and Stedman (2000) reported that in adult birds, blood lymphocytes probably arise mostly from peripheral lymphoid tissues including the spleen, caecal tonsils and other gut-associated lymphoid tissue. The incidence of promonocytes, mitotic cells, plasma cells and osteoclasts were comparable to other domestic species (Weiss and Wardrop, 2010).

Unlike mammalian platelets, which are cytoplasmic fragments of megakaryocytes, avian thrombocytes are derived from mononuclear precursor cells (Campbell and Coles, 1986; Bounous and Stedman, 2000).

The mean value for M/E ratio in the bone marrow of partridge was 1.33, which was comparable to that in duck (1.00) (Tadjalli *et al*., 1997), black head gull (1.23) (Tadjalli *et al*., 2002), camel (1.21) (Nazifi *et al*., 1998) and dog (0.75-2.5), cat (1.2-2.2), horse (0.5-.1.5), cattle (0.31-1.85) and sheep (0.77-1.68) (Weiss and Wardrop, 2010). However it differs from quail (0.37, Nazifi *et al*., 1999) and goat (0.69) (Weiss and Wardrop, 2010). The cellularity of the bone marrow smears was comparable to that of other domestic species (Andreasen *et al*., 1994; Spencer and Canfield, 1995; Nazifi *et al*., 1998; Nazifi *et al*., 1999; Weiss and Wardrop, 2010).

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