



## Routine red bone marrow smear in local breed calves at Basrah, Iraq

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### Abstract

The state of the peripheral blood reflects on bone marrow activity. Therefore, we need to know the normal red bone marrow, and it can be a reference for those interested in hematology. Calves of apparently healthy local breeds were used to examine the state of the bone marrow, take a sample of peripheral blood before slaughter, and then a bone marrow biopsy that was immediately after slaughter. The calves that showed typical values for the parameters of red blood cells and total and differential white blood cells were included in the study. The result of bone marrow smears for nine normal calves appeared to have a faint coloration of granulated myelocytic than erythroid cells. As well as the myeloid was less than the erythroid, and that M: E ratio was  $0.91 \pm 0.03$ . The erythroid series, Rubriblast, and Rubricyte were present as the most common progenitor red cell, including basophilic, polychromic, and normochromic cytoplasm, metarubricyte as a reduced nucleus. The myeloid series: Myeloblast, myelocyte was the most common progenitor white cells with granulation as basophilic, eosinophilic and neutrophilic within their cytoplasm, metamyelocyte with shrunken nuclei and band cell with horseshoe nucleus. They rarely found the megakaryocyte, enormous multinucleate cell.

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### Introduction

The bone marrow is a dynamic organ capable of structural and functional remodeling in response according to illnesses, and variations in demand for the production of red blood cells (RBCs), white blood cells (WBCs), and platelets (1,2). Moreover, bone marrow tissue lies in the central cavities of axial and long bones that comprise a sinusoidal system, hematopoietic cells, adipose tissue, supporting reticular cells, and an extracellular matrix (3,4). The RBCs, WBCs, and platelet cells evolved from the potential stem cells in the red marrow, which can differentiate into several cell series called erythroid and myeloid series (3,5). The value of red bone marrow elements distinguishes of disease status from peripheral blood counts such as leukemia, congenital hematopoietic defect, and cytological responses for bone marrow (6,7). Most endemic diseases in Iraq such as anaplasmosis, babesiosis and theileriosis (8,9), and other disease states associated with

anemia (10,11), may also be related to a defect in erythropoiesis and myelopoiesis (12,13), which can be characterized by a reduction in the levels of erythrocytes count, and of their other parameters in the blood periphery also dysplasia in one or more hematologic cell lines in the bone marrow (14,15). Authors assure that blood-borne diseases accompanied by diminished granulopoiesis and megakaryocytopoiesis in the bone marrow in the infected animal also the erythroid suppression, lymphocytic, monocytic, and macrophage hyperplasia are also found (3,16,17). At the same time, essential studies by Iraqi researchers are adapted to use bone marrow material for treatment in animals with no respect for average values of bone marrow activity (18,19). However, there is no previous study on local calves, so this study aimed to identify the red bone marrow cells in clinically normal calves, counting the myeloid: erythroid ratio and the differential count of myeloid and erythroid series.

## Materials and methods

### Animals

Anine local breed calves, aged between 3-4 months, they have chosen from the slaughterhouse of Basrah. Clinical examination performed according to Jackson and Cockcroft (20) to rule out those abnormal calves from the study. Samples of bone marrow biopsy was taken from the 4<sup>th</sup> sternebrae and preserved in sodium citrate (21,22). Then after making a bone marrow smear stained by Giemsa (23). Also count M: E ratio and differential erythroid and myeloid series (24). Collected blood samples from the jugular vein, and kept them in an EDTA tube to estimate blood parameters (25). All laboratory work occurred in the clinical pathology laboratory, College of Veterinary Medicine, University of Basrah. Statistics included the mean and standard error have done as in Andrade (26).

### Ethical approve

The research was approved by the college of veterinary medicine, University of Basrah, according to research ethics unit report no. 7/37, in 2022.

### Results

In the present study, we chose the normal calves; they were healthy for gross lesions following the exclusion of the abnormal cases. The blood periphery examination showed the RBC parameters' appearance value (Table 1). Also, for parameters of WBC are in table 2.

Table 1: The red blood cell parameters of local calves (n=9) in Basrah

Parameters	Mean±SE
RBC (10 <sup>12</sup> /L)	7.74±0.36
Hb (g/L)	105.3±3.01
PCV (%)	0.30±0.08
MCV (fL)	40.09±1.08
MCH (Pg)	13.73±0.35
MCHC (g/dL)	34.30±0.66

Table 2: Shows the total white blood cell and absolute differential counts of local calves in Basrah

Parameters	Mean±SE (x10 <sup>9</sup> /L)
Total WBC	9.31±0.57
Lymphocytes	4.56±0.38
Monocyte	0.85±0.14
Neutrophil	3.30±0.26
Eosinophils	0.30±0.05
Basophil	0.02±0.01

The bone marrow smears from biopsy samples immediately after the slaughtering of calves showed

differential myelocytic and erythrocytes counting as in tables 3. Moreover, myeloid: the erythroid ratio was 0.91±0.03 (Table 3). The characters of differential bone marrow cells as in figure 1, combined erythrocytic and myelocytic series. The erythrocytic series included Rubriblast (characterized by blue cytoplasm and dark nucleus). The Rubricyte contains basophilic, polychromic, and normochromic cytoplasm. Metarubricyte that had shrunken nuclei may tack kidney shape (Figure 1).

Table 3: The percentage of differential myelocytic series of local calves in Basrah

Series	Type (%)	Mean ± SE
Myeloid	Myeloblast	7.44±1.01
	Myelocyte	38.00±2.78
	Metamyelocyte	19.67±1.24
	Band cell	33.44±2.08
Erythroid	Rubriblast	6±1.14
	Rubricyte	40.22±4.35
	Metarubricyte	23.56±4.47
no. = 9	M:E ratio	0.91±0.03

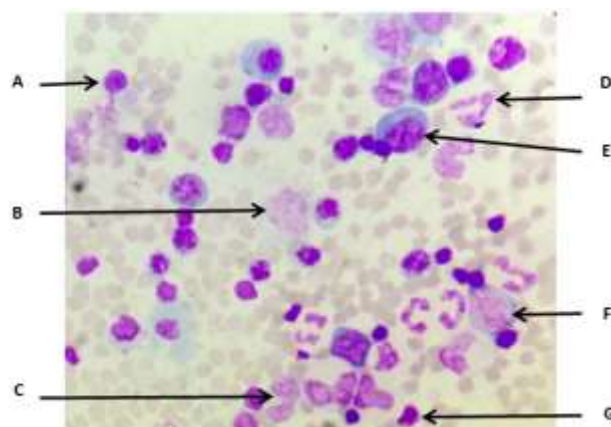


Figure 1: Bone marrow smear from local calves. A; rubricyte, B; myelocyte, C; metamyelocyte, D; band cell, E; rubriblast, F; myeloblast, G; metarubricyte. Giemsa stain, X1000.

The Myelocytic series was in figures 1 had faint coloration than erythrocytic cells which include: Myeloblast as similar to Rubriblast but had weak color, myelocyte that had granules in their cytoplasm (basophilic, eosinophilic, and neutrophilic). Metamyelocytes had shrunken nuclei and cytoplasmic granules (basophilic, eosinophilic, and neutrophilic). A horseshoe-shaped nucleus manifested the Band cell as in figure 1. We found megakaryocytes only in one calve bone marrow smear, large multinucleated cells (Figure 2).

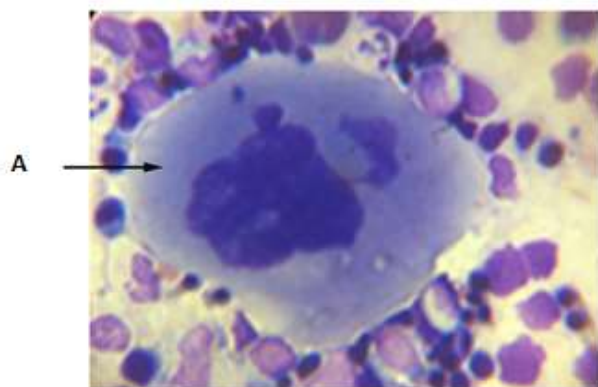


Figure 2: Bone marrow smear from local calves. The megakaryocyte appears as most significant cell. Giemsa stain, X1000.

### Discussion

The examination of locally bred calves before their slaughtered was normal clinically, and the gross pathological examination was at healthy limitation following slaughter (20,27). According to several authors, the exploration of blood parameters for RBC and WBC revealed normal levels (28-30), except MCV mean, which was higher than Mohri (29) observed in related calve age. On the other hand, the present MCV level was less than adult cows according to reference data, that cows blood estimated MCV by mean of 50 fL and can reach the maximum level of 58.7 fL (30,31). The calves show multiple changes in their blood parameters during the first six months of their life (32,33). Therefore, we chose 3-4 months age calves to estimate bone marrow.

The characters of the erythroid series were; Rubriblast with marked blue cytoplasm, while rubricyte has basophilic and or red cytoplasm because of incorporation of hemoglobin (1). Moreover, metarubricyte had a small clumped nucleus because of a reduction in its size and deep red cytoplasm that were also described (6,34). The myeloid series description was fainter than Rubriblast in staining (24) and included; Myeloblast, myelocyte had granulated cytoplasm, metamyelocyte with a reduced nucleus, and band cell with characters of the horseshoe nucleus, and that was like previous observations (1). We showed the differentiated rubricate and myelocyte in a high percentage, and such results were reported because several mitotic divisions included more maturation until they passed to the following progenitor stage (35,36). We did not include the other advanced maturation stages for the myelocytic and erythrocytic series to avoid mistakes with peripheral blood elements that may enter the bone marrow smear during the sample collection procedure (24,37). The peripheral blood is mixed with bone marrow specimens at the collecting sample. The myeloid: erythroid ratio by mean  $0.91 \pm 0.03$  indicates that dark erythrocytic cells are higher than faint myeloid cells

found in the bone marrow smears, which was pointed out in normal animals (5,38). The young, healthy animals had the activity of erythroid division more than myeloid when suggesting a normal blood periphery status (39,40). The megakaryocyte, a progenitor cell for the platelets (41), was rarely seen in the present study, which explained that megakaryocytes are often not outwarded in bone marrow biopsy (5,42,43).

### Conclusion

The young calves 3-4 months had active bone marrow progenitors with a high proportion of erythrocytic cells than myelocytic cells. Also, the rubricytes with the dark nuclei and myelocytes with light nuclei were most common in the red bone marrow.

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### Conflict of interest

Authors declared that there is no conflict of interest.

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خزعة نقي العظم بعد الذبح مباشرة . وأدخلت في الدراسة العجول التي أظهرت قيما طبيعية لمتنابئات كريات الدم الحمر، وتعداد كريات الدم البيض الكلية والتفاضلية. لذلك تم اختيار تسعة عجول. أظهرت نتائج لطاخات نقي العظم للعجول التسعة الطبيعية تلون خافت للخلايا النقية المكونة للبيض المحببة مقارنة لمكونة الحمر. وان نسبة الخلايا المكونة للبيض أقل من المكونة للحمر وبمعدل نسبة ام: اي ٩١،٠ لصالح الحمر. وكانت السلسلة المكونة للحمر من الأرومة المضرجة، والمضرجة وهي أكثر تواجدا بوصفها الخلية الحمراء الأكثر انشطارا وتضمنت ذات السيتوبلازم القاعدي ومتعدد الألوان وسوي الصباغ، وخلية ما بعد الأرومة الحمراء تحتوي على نواة مختزلة. السلسلة المكونة للبيض كانت: الأرومة النقية، والخلية النقية وهي الخلايا البيضاء السلفية الأكثر شيوعا والتي تحتوي على حبيبات في الهولي مثل الحبيبات القعدة والحمضة والعدلة، والخلايا ما بعد النقية ذات النوى المنكمشة والخلية الشريطية مع نواة شبه حدوة الحصان. ونادرا ما وجدت خلية النواء، وهي خلية متعددة النوى كبيرة جدا.

## لطاخه نقي العظم الأحمر الروتيني في سلالات العجول المحلية في البصرة، العراق

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### الخلاصة

تتعرض حالة الدم المحيطي على نشاط نقي العظم، لذلك نحتاج إلى معرفة نقي العظم الأحمر الطبيعي، ويمكن أن يكون مرجعا للمهتمين بعلم الدمويات. استخدمت عجول من سلالات محلية سليمة ظاهريا لفحص حالة نقي العظم ، و أخذت عينة من الدم المحيطي قبل الذبح، وأيضا