

Morphological characteristics of blood cells in clinically normal adult llamas (*Lama glama*)

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ABSTRACT

Morphological characteristics, including numeration, differentiation and features of blood cells were determined in ten male and ten female clinically normal adult llamas. Haematological parameters included: erythrocyte count, haemoglobin concentration, packed cell volume, erythrocyte indices, reticulocyte count, platelet count, total leukocyte count and differential leukocyte count. It appeared that llama haemogram was characterized by the presence of numerous but small erythrocytes, high total leukocyte count and a high number of mainly immature eosinophils. Unique cellular morphological characteristics commonly observed in May-Grunwald-Giemsa stained blood smears were: folded erythrocytes, Cabot's rings, hypersegmented neutrophil nuclei, granular lymphocytes and immature eosinophils.

Key words: haematological parameters, morphology of blood cells, *Lama glama*, Cabot's rings, granular lymphocytes

Introduction

South American camelids have evolved at elevations of 4000 meters or more and are therefore well adapted to high altitudes. The unique morphological features of llama erythrocytes play a vital role in their ability to transport oxygen to the tissue adequately at these elevations without increasing their erythrocyte mass. The leukogram is characterized by a high total leukocyte count accompanied by high neutrophil and eosinophil counts (REYNAFARJE et al., 1968). In the last decades llamas were introduced

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to new environments, at sea-level, in many countries of North America, Europe, Asia and Africa (FOWLER and ZINKL, 1989; AL-ANI et al., 1992; SMITH, 1993; TAIT et al., 2002; AL-IZZI et al., 2004). Adaptation to these environments did not change the blood constituents of llama (FOWLER and ZINKL, 1989; AL-IZZI et al., 2004). The present study was designed to describe morphological characteristics of erythrocytes, platelets and leukocytes, including their counts in clinically normal adult llamas.

Materials and methods

The present study was conducted on ten male and ten female clinically normal adult (more than 2 years old) llamas (*Lama glama*). The llamas were on routine anthelmintic management programs and were free from ectoparasites and blood parasites. The llamas were kept in a fenced partially shedded open yard at Surman Park in Libya. They were provided with water, green food, concentrates and blocks containing minerals and essential trace elements.

Five mL of blood was collected from the jugular vein of each animal into plastic tubes containing EDTA. Haemoglobin concentration, platelet count and total leukocyte count were determined using Sysmex™ Model NE-1500 (Toa Medical Electronics Co. Ltd, Japan). Erythrocytes were counted using Neubauer haemocytometer according to JAIN (1986). Microhaematocrit centrifuge was employed to measure packed cell volume (PCV). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated as mentioned by JAIN (1986). Reticulocyte and Cabot's ring erythrocyte counts were done by differentiation 500 erythrocytes in brilliant cresyl blue stained blood smear. Blood smears were stained with May-Grunwald-Giemsa stain and 200 leukocytes were differentiated in one smear prepared from each animal. In addition, 100 lymphocytes, 100 eosinophils and 100 neutrophils were counted in each stained blood smear to determine the percentages of granular lymphocytes, eosinophils at different stages of maturation, neutrophilic nuclear hypersegmentation, and the presence of Barr bodies. An eyepiece micrometer was used to measure the diameters of the cells.

Results

The mean, standard deviation and range values of haematological parameters in adult llamas are presented in Table 1. It appeared that those parameters were characterized by high erythrocyte, leukocyte, neutrophil and eosinophil counts.

Erythrocytes. Erythrocytes were numerous (10.3 to $15.0 \times 10^6/\mu\text{L}$), small ($7.32 \pm 0.95 \times 3.9 \pm 0.52 \mu\text{m}$), flat, elliptical, lacked central pallor and their outlines were less distinct than usual (Fig. 1). Their diameters varied from 5.74 to $11.48 \mu\text{m}$ and 3.43 to $5.74 \mu\text{m}$

for the greater and lesser diameters, respectively. A few red blood cells were folded and slight anisocytosis was common. The calculated MCV was low, while MCHC was high. Reticulocytes and nucleated erythrocytes were larger and more rounded than mature ones. Faint lines in the form of a ring bordering the cell membrane or figure eight occupying the whole cell were observed in 2.7% of the llamaid erythrocytes in brilliant cresyl blue stained blood smears (Fig. 2). These lines were identified as Cabot's rings. Polychromatic rubricytes, metarubricytes (0.8/100WBC) and cells containing Howell-Jolly bodies were observed in May-Grünwald-Giemsa stained blood smears. Distribution of these cells was 20%, 74% and 6%, respectively, among 100 nucleated erythrocytes.

Table 1. Haematological parameters in clinically normal adult llamas

Parameter	Mean \pm SD n = 20	Range n = 20
Erythrocytes ($\times 10^6/\mu\text{L}$)	12.3 \pm 1.5	10.3 - 15.0
Haemoglobin (g/dL)	13.7 \pm 1.4	12.0 - 17.5
PCV (%)	31.4 \pm 3.1	28.0 - 35.0
MCV (fL)	25.5 \pm 2.8	21.1 - 31.0
MCH (pg)	11.1 \pm 1.1	9.6 - 13.6
MCHC (g/dL)	43.6 \pm 2.8	38.6 - 48.0
Nucleated erythrocytes (/100 WBC)	0.8 \pm 0.9	0.0 - 3.0
Reticulocytes (%)	0.4 \pm 0.1	0.2 - 0.6
Cabot's rings (%)	2.7 \pm 1.3	1.0 - 5.0
Platelets ($\times 10^5/\mu\text{L}$)	4.1 \pm 1.7	2.1 - 8.0
Leucocytes ($\times 10^3/\mu\text{L}$)	15.6 \pm 4.0	9.7 - 22.0
Neutrophils ($/\mu\text{L}$)	10.125 \pm 2.716	6.750 - 14.750
Band neutrophils ($/\mu\text{L}$)	58 \pm 94	0 - 350
Lymphocytes ($/\mu\text{L}$)	3.390 \pm 1.167	1.360 - 5.750
Monocytes ($/\mu\text{L}$)	281 \pm 176	0 - 690
Eosinophils ($/\mu\text{L}$)	1.735 \pm 914	876 - 4.000
Basophils ($/\mu\text{L}$)	11 \pm 33	0 - 114

Platelets. Platelet count ranged from 2.1 to 8.0 $\times 10^5/\mu\text{L}$. They were small, with a mean diameter of 2.5 μm and a range of 1.72 to 4.59 μm . Platelets were round to irregular in shape, with pinkish cytoplasm containing azurophilic granules (Fig. 1).

Leukocytes. Total leukocyte count in llamas was high (15.6 $\times 10^3/\mu\text{L}$) accompanied by high neutrophil (10.125/ μL) and eosinophil (1.735/ μL) counts. Unique morphological features were observed in neutrophils, lymphocytes and eosinophils.

Neutrophils. Neutrophil count ranged from 6,750 to 14,750/ μ L. Most of the neutrophil nuclei were distinctly segmented, connected by fine filaments or constrictions. Nuclear hypersegmentation (6 lobes or more) was observed in some neutrophils (1 to 21%) (Fig. 1). A well-defined round head appendage of the female sex chromatin (Barr body) was seen attached to the nucleus of some female neutrophils (1 to 9%). However, smaller minor clubs or nodules were found connected to the nuclei of neutrophils of some male llamas. The cytoplasm of mature neutrophils contained a few faintly pink granules.

Table 2. Eosinophil and lymphocyte differential counts in clinically normal adult llamas

Cell Type	Mean \pm SD (%) n = 20	Range (%) n = 20
Eosinophilic myelocytes	0.5 \pm 0.9	0.0 - 4.0
Eosinophilic metamyelocytes	12.0 \pm 4.6	4.0 - 19.0
Band eosinophils	53.0 \pm 8.1	32.0 - 65.0
Segmented eosinophils	28.0 \pm 8.8	16.0 - 51.0
Ring eosinophils	6.5 \pm 2.8	1.0 - 10.0
Granular lymphocytes	4.9 \pm 4.2	1.0 - 19.0
Agranular lymphocytes	95.1 \pm 4.2	81.0 - 99.0

Lymphocytes. Lymphocyte count ranged from 1,360 to 5,750/ μ L. As in other animal species, llama blood contained small, medium and large lymphocytes. Small lymphocytes had round, intensely stained nuclei, sometimes with an indentation. The cytoplasm of

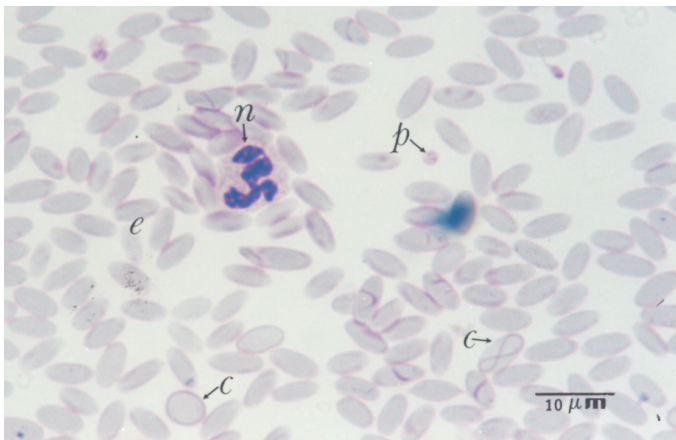


Fig. 1. Erythrocyte (e), Cabot's rings inside erythrocytes (c), platelet (p) and neutrophil with hypersegmented nucleus (n). May-Grünwald-Giemsa stain; \times 1000.

small lymphocytes was light blue, scanty and appeared as a thin rim around the nucleus. Medium lymphocytes were larger than small lymphocytes, with more abundant cytoplasm. The nuclei stained lighter and the chromatin appeared less dense. Large lymphocytes exhibited a low nuclear to cytoplasm ratio and the nuclei were either spherical or indented with fine chromatin. The cytoplasm stained homogeneously blue.

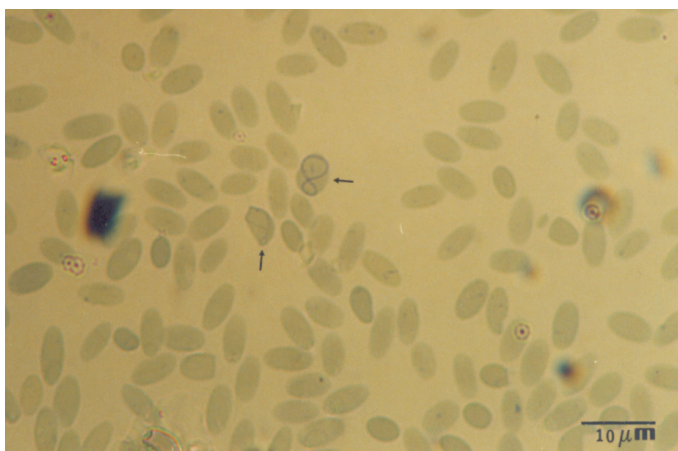


Fig. 2. Cabot's rings inside erythrocytes (arrows). Brilliant cresyl blue stain; $\times 1000$.

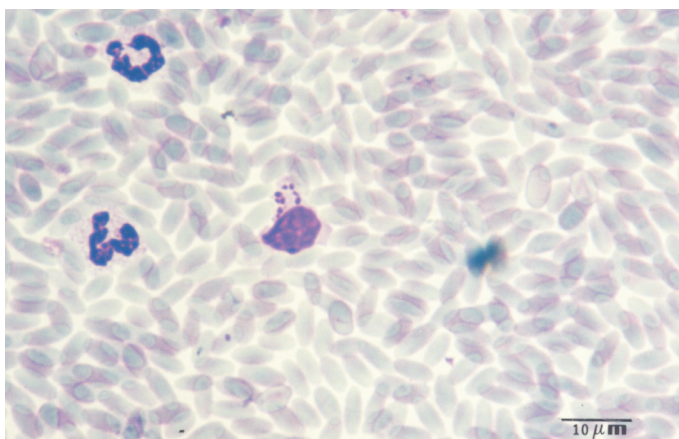


Fig. 3. Large granular lymphocyte (arrow) with fine nuclear chromatin and abundant cytoplasm. May-Grünwald-Giemsa stain; $\times 1000$.

A subpopulation of granular lymphocytes was observed in stained blood smears (Fig. 3). These cells constituted from 1 to 19% of the lymphocyte count (Table 2). The cytoplasm of granular lymphocytes contained from 2 to 20 dark, purplish-blue azurophilic granules. The size of these granules varied from tiny, dust-like, to large granules. They were highly pleomorphic with round, oval and rod shapes. Small (6.89 to 8.00 μm), medium (8.04 to 8.61 μm) and large (9.18 to 14.92 μm) lymphocytes represented 12.1%, 11.2% and 76.7% of the granular subpopulation, respectively. The mean number of granules in the cytoplasm of large lymphocytes (7 granules/cell) was higher than those in medium (5 granules/cell) and small (4 granules/cell) lymphocytes, but there was weak correlation (correlation coefficient = 0.31) between the number of granules and the size of the lymphocyte.

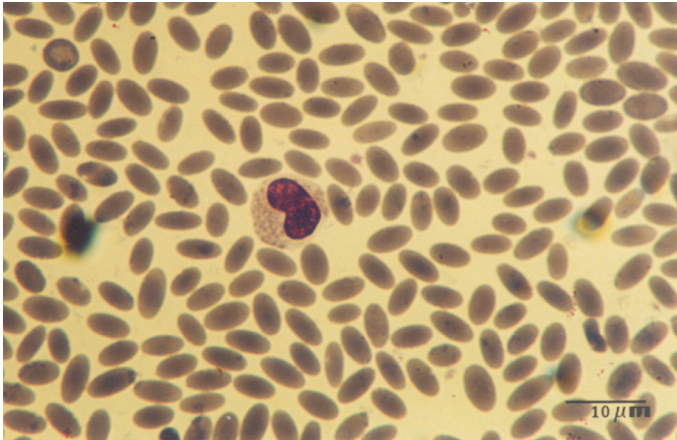


Fig. 4. Eosinophilic metamyelocyte with kidney shape nucleus and a few cytoplasmic granules. May-Grünwald-Giemsa stain; $\times 1000$.

Monocytes. Monocyte count ranged from 0 to 690/ μL . These cells were large, with deeply indented, horseshoe- or kidney-shaped nuclei, blue-gray cytoplasm, and multiple discrete cytoplasmic vacuoles.

Eosinophils. The most outstanding feature of the llama leukogram was the high number of mainly immature eosinophils (876 to 4,000/ μL) including myelocytes, metamyelocytes and bands (Fig. 4). In addition, eosinophils with ring-shaped nuclei (doughnuts) were observed in stained blood smears (Fig. 5). Most of the segmented eosinophils contained bilobed nuclei, although trilobed nuclei were found in very few of them. Differential eosinophil count revealed that 0.5%, 12.0%, 53.0%, 28.0% and 6.5% were myelocytes,

metamyelocytes, bands, segmented and ring eosinophils, respectively (Table 2). Small, irregular, reddish-orange granules incompletely filling the cytoplasm were seen in llama eosinophils.

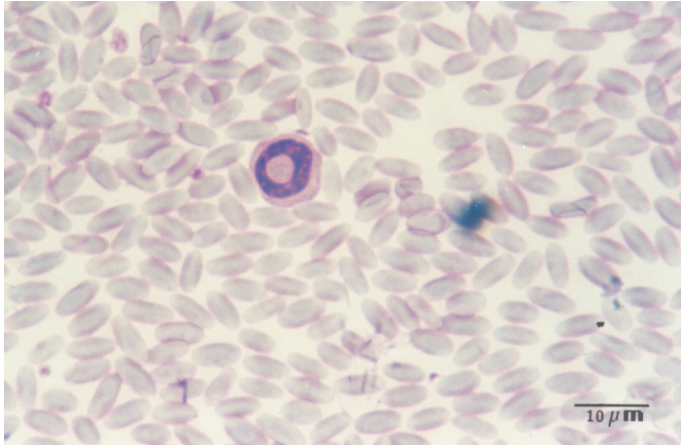


Fig. 5. Eosinophil with ring-shaped nucleus. May-Grunwald-Giemsa stain; $\times 1000$.

Basophils. Basophil count ranged from 0 to $114/\mu\text{L}$. Basophils had poorly segmented nuclei and multiple small, purple cytoplasmic granules. The granules partially obscured the nucleus.

Discussion

The mean and range values of haematological parameters examined in this study were within the ranges established previously for clinically normal llamas (*Lama glama*) (AL-IZZI et al., 2004).

Llamoid erythrocytes were small ($7.32 \pm 0.95 \times 3.9 \pm 0.52 \mu\text{m}$), elliptical, flat and their counts obtained in the present study (10.3 to $15.0 \times 10^6/\mu\text{L}$) were higher than those in other domestic animals (FELDMAN et al., 2000). The flat shape and the presence of the few folded erythrocytes were attributed to the low thickness to diameter ratio of llama red blood cells (VAN HOUTEN et al., 1992). The small volume resulted in a high concentration of erythrocytes for any given PCV. The high MCHC of llama (38.6 to 48.0 g/dL) in comparison with those in other species might be due to the flat nature of the erythrocytes, which allowed more space for haemoglobin molecules to increase their efficiency for carrying oxygen at high altitude (HAWKEY, 1975). It was evident that llama bone marrow, unlike other ruminants except dromedary camels (MOORE, 2000), was normally releasing

immature erythrocytes, including polychromatic rubricytes, metarubricytes (0.8%) and reticulocytes (0.4%) into circulation. Cabot's rings appeared inside a few erythrocytes (1 to 5 %) of all llamas included in this study. Brilliant cresyl blue vital stain seemed to be more efficient in demonstrating these threads than May-Grunwald-Giemsa stain. These rings were also seen in the erythrocytes of normal Bactrian camels (HAWKEY, 1975; MOORE, 2000). The diagnostic significance of the presence of Cabot's rings is controversial. Some authors considered Cabot's rings as arginine-rich histones and non-haemoglobin iron resulting from abnormal histone biosynthesis in pernicious anaemia (LEE, 1999), while others mentioned that these rings were artifacts representing denatured membrane protein with no diagnostic significance (BEGEMANN and ROSTETTER, 1979; MOORE, 2000). In general, the unique elliptical, flat camelid erythrocytes facilitate their movement in capillaries at times of dehydration in arid areas, (SMITH et al., 1979; SMITH et al., 1980) minimizing the likelihood of sludging.

The characteristic morphological features of llama neutrophils were the hypersegmentation of some (1 to 21%) neutrophil nuclei and the presence of female sex chromatin in the nuclei of 1 to 9% of neutrophils. Similarly, nuclei of neutrophils in Bactrian camels were distinctly lobulated and hypersegmented (MOORE, 2000). Female sex chromatin was observed in neutrophils of various species, manifested as a "drumstick" lobe on the nucleus (KRAFT, 1957; HAWKEY, 1975; FELDMAN et al., 2000). Small minor clubs or nodules were seen on the nuclei of some male neutrophils and must be differentiated from the female sex chromatin.

In the current study, most of the leukocytes were neutrophils, which is a known finding in llamas and camels (FOWLER, 1998; MOORE, 2000), while in other ruminants lymphocytes are the predominant leukocyte. However, camelidae are known to be different from true ruminants in a number of important characteristics, which is not surprising since in a taxonomic sense they are not considered as ruminants, although they spend many hours each day ruminating (FOWLER, 1999).

Small, medium and large lymphocytes, morphologically similar to those in other ruminants (FELDMAN et al., 2000), were observed in llama blood smears examined in this study. Granular lymphocytes are those containing cytoplasmic azurophilic granules and are present as a subpopulation in nearly all animal species (FELDMAN et al., 2000). In llamas, such cells were confined to large lymphocytes only and comprised 7 to 29% of lymphocyte population (VAN HOUTEN et al., 1992). However, in the present study granular lymphocytes constituted 1 to 19% of the total lymphocyte count, and the azurophilic granules were found in small, medium and large lymphocytes. In humans, azurophilic granules have been recorded in both small and large lymphocytes (JUNQUEIRA et al., 1998). Although it is now recognized that not all granular lymphocytes are large or have abundant cytoplasm, most literature still refers to this population of lymphocyte as large

granular lymphocytes (WELLMAN, 2000). Functionally, large granular lymphocytes have natural killer and antibody-dependent cell-mediated cytotoxic activity (MIDDLETON, 1999).

The most outstanding feature of the llama leukogram was the high number of eosinophils. The eosinophil count (876 to 4,000/ μ L) was relatively high in comparison with those of other species. The eosinophilia in llama was attributed to the persistent sub-clinical endo- and ectoparasitism (ELLIS, 1982; HAWKEY and GULLAND, 1988; FOWLER and ZINKL, 1989). However, llamas used in the present study were on routine anthelmintic management programs and were free from ectoparasites, suggesting that eosinophilia might be a characteristic feature of this species. This finding was consistent with observations on parasite-free llamas (VAN HOUTEN et al., 1992; ABDOUSLAM et al., 2003). Eosinophil differential count revealed that most of these cells were immature, including myelocytes, metamyelocytes and bands, as well as eosinophils containing ring-shaped nuclei. Eosinophils with hyposegmented band to bilobed nuclei accompanied by high cell count were recorded in healthy adult llamas (VAN HOUTEN et al., 1992). The mechanism of the release of immature eosinophils from the bone marrow to the circulation under normal conditions is unknown. Monocyte and basophil morphology was similar to that of other mammals (HAWKEY and GULLAND, 1988).

Unique morphological characteristics of llama blood cells commonly observed and interpreted as normal included folded erythrocytes, Cabot's rings, hypersegmented neutrophil nuclei, granular lymphocytes and hyposegmented eosinophils. In addition to the normal values of haematological parameters, these unique cellular morphological features can be used for interpretation of haematological results.

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SAŽETAK

U 10 muških i 10 ženskih klinički zdravih odraslih ljama određivane su morfološke značajke, broj, razlike i osebnost krvnih stanica. Određivan je broj eritrocita, koncentracija hemoglobina, hematokrit, obilježja eritrocita, broj retikulocita, broj trombocita, ukupni broj leukocita i diferenciranost leukocita. Čini se da je za hemogram ljama karakterističan velik broj eritrocita male veličine, zatim velik ukupni broj leukocita i velik broj uglavnom nezrelih eozinofila. Jedinstvene morfološke značajke stanica, promatrane u krvnim razmascima obojenima po May-Grünwald-Giemsu, bile su naborani eritrociti, Cabotovi prsteni, hipersegmentirane jezgre neutrofila, granulirani limfociti i nezreli eozinofili.

Ključne riječi: hematološki pokazatelji, morfološka krvnih stanica, *Lama glama*, Cabotovi prsteni, granulirani limfociti
