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ORIGINAL RESEARCH

The influence of age, sex and altitude on the morphometry of red blood cells in bovines

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Abstract

Aim: This study was conducted on local bovine breed (black and white breed) in the east and the south of Algeria in order to reveal the influence of age, sex and altitude on the morphometry of red blood cells.

Materials and methods: The animals were divided into three equal groups at different altitudes while the animals of each group include adult males, adult females, young males, young females; and live at the same altitude in the following regions: region A, Wilaya of Batna; region B, Wilaya of Biskra; and region C, in the Wilaya of El-Oued. Blood samples were collected from the jugular vein and blood smears were immediately performed. The smears were stained according to the May-Grundwald-Giemsa method. The morphometric study was achieved using with a graduted ocular and a micrometric slide with an immersion optic microscope at a grossissement of 100. Statistical analysis was undertaken using the T student test.

Results: The results showed a significant influence of age on the morphometry of red blood cells, the young bovine's red blood cells are significantly larger than the adults erythrocytes in the three regions. Indeed, sex have a significant influence on the size of red blood cells, the erythrocytes of males are significantly larger than the females erythrocytes in the three region. However, concerning the altitude, the study showed a significant difference between the Wilaya of Batna group and the two other groups, the bovine's red blood cells of Batna Wilaya are significantly larger than the bovine's red blood cells of Biskra and El-Oued groups.

Conclusion: Therefore, age, sex and altitude have profound effect on the morphometry of red blood cells and careful attention must be observed in studying and interpretation of anemic syndromes.

Keywords: age, altitude, black and white, bovines, morphometry, sex

Introduction

The mature red blood cell (RBC) of the adult bovine is biconcave in shape [1], has a width of 5-6µm, has minimal central pallor and relatively long lifespan of approximately 130 days [2]. Red blood cell shape is relatively uniform; rouleaux formation of bovine erythrocytes is unusual unless inflammatory proteins are increased [3]. Polychromatophils are generally absent from the blood of animal adult cattle [4]. However, when severe anemia occurs, reticulocytosis appears in peripheral blood in association with macrocytosis, polychromasia, and basophilic stippling of red blood cells, indicating accelerated erythropoiesis [5]. Age, sex, exercise and emotional state are variables to be considered when establishing references values in domestic cattle [6]. Bulls have appreciably greater red blood cells counts than cows; breed differences have been reported for beef cattle, which have greater red blood cells values than dairy cattle breeds; lactating cows have consistently lower red blood cells values than non-lactating cows; altitude polyglobulia (increased red blood cells count at high altitude) is well documented and extensively studied [6]; while few references were found on blood cell morphometry.

This study was carried out on local Black and White bovines breed and it aims to investigate the influence of three factors: age, sex and altitude on red blood cells morphometry to prevent any possible confusion of anemic syndromes.

Materials and Methods

Study area and animals: Three regions of the east and the south of Algeria at different altitudes were chosen namely: Region A (Batna), in the east at with 1000m above sea level; Region B (Biskra), in the south-east at 110 meters above the sea-level; and Region C (El-Oued), in the south at the same level as the sea. Forty four subjects were taken for each area of study; clinically healthy animals were divided according to their age and sex in four subgroups as follows: 15 adult males, 15 non pregnant adult females, 7 young males and 7 young females.

Ethical approval: Adequate measures were taken to minimize pain or discomfort in accordance with the International Animal Ethics Committee. The study was approved by the committee framed for the research by the university authority. During the study visits, the researchers introduced themselves and explained the

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Table-1. Influence of age on the size of bovine erythrocytes (expressed in µm)

Animals and region	Adult (M ± S.D)	Young (M ± S.D)
Bovines (N = 14)		
Batna	5,26 ± 0,33	5,62 ± 0,28**
Biskra	4,58 ± 0,37	5,03 ± 0,32**
El-Oued	4,62 ± 0,32	5,11 ± 0,25***
Bovines males (N = 07)		
Batna	5,55 ± 0,12	5,88 ± 0,10***
Biskra	4,91 ± 0,16	5,31 ± 0,13***
El-Oued	4,91 ± 0,15	5,34 ± 0,09***
Bovines females (N = 07)		
Batna	4,97 ± 0,14	5,37 ± 0,07***
Biskra	4,25 ± 0,15	4,75 ± 0,17***
El-Oued	4,34 ± 0,09	4,88 ± 0,06***

M: Mean ; S.D: Standard-Deviation ; ** : $p < 0,01$; *** : $p < 0.001$

Table-2. Influence of sex on the size of Bovine erythrocytes (expressed in µm)

Animals and region	Males (M ± S.D)	Females (M ± S.D)
Bovines (N = 22)		
Batna	5,62 ± 0,22***	5,11 ± 0,21
Biskra	5,05 ± 0,23***	4,40 ± 0,28
El-Oued	5,05 ± 0,23***	4,51 ± 0,28
Bovines adults (N = 15)		
Batna	5,50 ± 0,13***	4,99 ± 0,12
Biskra	4,92 ± 0,14***	4,23 ± 0,13
El-Oued	4,92 ± 0,13***	4,34 ± 0,14
Bovines young (N = 07)		
Batna	5,88 ± 0,10***	5,37 ± 0,07
Biskra	5,31 ± 0,13***	4,75 ± 0,17
El-Oued	5,34 ± 0,09***	4,88 ± 0,06

M: Mean ; S.D: Standard-Deviation ; *** : $p < 0.001$

Table-3. Influence of altitude on bovine red blood size (expressed in µm)

Animals and region	**Batna** (M ± S.D)	**Biskra** (M ± S.D)	**El-Oued (M ± S.D)**
Bovines (N = 44)	5,36 ± 0,33***	4,72 ± 0,41	4,78 ± 0,37
Adults (N = 30)	5,24 ± 0,28***	4,58 ± 0,37	4,63 ± 0,32
Adultes males (N = 15)	5,50 ± 0,13***	4,92 ± 0,14	4,92 ± 0,13
Adultes females (N = 15)	4,99 ± 0,12***	4,23 ± 0,13	4,34 ± 0,14
Young (N = 14)	5,62 ± 0,28***	5,03 ± 0,32	5,11 ± 0,25
Young males (N = 07)	5,88 ± 0,10***	5,31 ± 0,13	5,34 ± 0,09
Young females (N = 07)	5,37 ± 0,07***	4,75 ± 0,17	4,88 ± 0,06
Males (N = 22)	5,62 ± 0,22***	5,05 ± 0,23	5,05 ± 0,23
Females (N = 22)	5,11 ± 0,21***	4,40 ± 0,28	4,51 ± 0,28

M: Mean ; S.D: Standard-Deviation ; *** : $p < 0.001$

objective and; methodology of the study to all cattle breeders.

Blood samples: After disinfecting of the sampling area, blood samples were taken from the jugular vein [7]; smears were confectioned on microscope slides just after venupuncture without anticoagulants which may interfere and induce some cytoplasmic and morphometric cell changes and on the extreme provoke degranulation of some blood cells [8]. Slides are precisely identified (order number, region) and classified in slides racks which are equipped with an information sheet including: region, sex and age of the animals following the order number mentioned on the slide; these information are recorded immediately after each sampling and smear realization.

Blood smears staining and morphometric study: In laboratory smears were stained by the May-Grundwald-Giemsa method [8]; the diameter of erythrocytes is measured by combination of an ocular micrometer and an objective micrometer using an optical microscope at a 100 magnification immersion (x100) [9]. To each subject, 100 erythrocytes were measured in four different fields in the area of ideal thickness of the smear, with 25 red blood cells per field, and then we determine the average of 100 erythrocytes for all individuals in the three regions [10].

Statistical analysis: Data analysis was done with the help of Student T test for comparison of two means, using STATITCF (vesion 5.0).

Results

Influence of age: The results are shown in Table-1, indicate the influence of the age of cattle (male and female) on the size of red blood cells which are significantly larger in young cattle than in adults ($p < 0.01$ and $p < 0.001$) in the three regions; the values are in the range of international standards.

Influence of sex: The Table-2 shows the influence of cattle sex (adult and young) on the size of red blood cells with an advantage in males compared to females. It should be noted that all the differences are significant ($p<0.001$). The results obtained are in the range of physiological norms cited by different authors.

Influence of altitude: Red blood cells are significantly larger in Batna's Cattle compared to those of Biskra and El-Oued ($p<0.001$); however, there is no significant difference when comparing Biskra's animal erythrocytes with those of El-Oued; the observed results remain still within the range of international standards (Table-3).

Discussion

Influence of age: According to Schalm and Carlson [11], Harvey et al. [12], Meinkoth and Clinkenbeard [13] and Harvey [14], the fetal erythrocytes are larger than those of adults. During gestation and at birth, the erythron compartment increase, at birth 9% of the red blood cells are reticulocytes [15]. Fetal calf red blood cells are less fragile and larger than adult bovine red blood cells [16]. The increasing of erythrocytes diameter in young cattle, observed in our study could be interpreted by the persistence of red blood cells after birth formed during embryonic life, and the decreasing of the diameter by the stem cell adaptation to new conditions of life after parturition.

Influence of sex: Size increase in red blood cells of

males compared to females could a bovine property or linked to our local breed. Extended studies to other breeds and species must be conducted.

Influence of altitude: The size of erythrocytes is conditioned by the stage at which they are released into the circulation from the bone marrow [17]. In normal animals, erythropoiesis and red blood cells mas are regulated by cellular oxygen level. During erythropoiesis, cell size decreases from the pro-erythroblast to erythrocyte [18]. This increase in the erythrocytes diameter at high altitudes showed in our study could be interpreted by hypoxia that occurs in high altitudes and which leads to the reduction of erythropoiesis time, by passing some stages of maturation to give larger erythrocytes.

Conclusion

The morphometric study of bovine red blood cells performed on 132 blood smears according to age, sex and altitude has allowed to show that: red blood cells are significantly larger in younger than adult animals; the cells are slightly larger in males than in females; the cattle reared at high altitude (over 1000m) in the region of Batna and which have their erythrocytes larger than those of compared animals reared in low altitude in the regions of Biskra and El-Oued. These results could serve as a basis for the diagnostic interpretation of anaemic syndromes in veterinary medicine especially concerning normocytic, macrocytic, and microcytic anemia. Further studies are highly recommended to show the exact effect age, sex and altitude on red blood cells morphology.

Authors' contribution

All authors contributed equally. All authors read and approved the final manuscript.

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Competing interests

Authors declare that they have no competing interest.

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