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Prevalence and Age-Related Patterns of Erythrocyte Morphological Abnormalities in Dairy Cows from Tiaret, Algeria

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Abstract: Our study was conducted between January 2025 and May 2025 on local dairy cows raised in the Tiaret region of Algeria. These cows were divided into three age groups: ≤ 3 years, 3 to 6 years, and >6 years. The main objective of this work was to determine the prevalence of all types of morphological abnormalities affecting erythrocytes and to evaluate the effect of age on the prevalence of these abnormalities. A total of 50 blood samples were used to perform a complete blood count and blood smears. This study revealed average values of erythrocyte lineage blood counts within the references cited in the literature and an overall prevalence of morphological abnormalities of erythrocytes in 68% of the cases studied, with a predominance of shape abnormalities (58.82%) and size abnormalities (52.94%). We also observed that young cows aged < 3 years were particularly prone to these anomalies. We also found that the most frequent shape abnormalities were acanthocytes which were present in 50% of all the cows studied. 30% of young cows and 20% of cows aged between 3 to 6 years showed acanthocytes. Dacryocytes, were recorded 30% in all cows, with a very high percentage (25%) in young cows. Anisocytes, which show size anomalies, were present in cows of all age groups. Color abnormalities and inclusion abnormalities were very rarely observed. These results therefore highlight the influence of age on the erythrocyte profile of cows and emphasize the importance of blood monitoring to improve their health and productivity.

Key words: Dairy cows, erythrocytes, abnormalities, hemogram, age.

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One of the most important functions of blood tissue is to maintain the physiological balance of the organism. Several key blood indicators allow for the assessment of this balance and the determination of the animal's adaptation to its environment. These indicators vary depending on several factors, such as age, reproductive status, sex, breed, and even the animal's weight (Hariche, 2021; Boudebza *et al.,* 2014; Vojta *et al.,* 2011). In veterinary medicine, essential blood tests are also employed to diagnose several ruminant ailments. These tests assist inmonitoring the overall health of the herd and enable early detection of certain diseases (Machado *et al.,* 2024; Yang *et al.,* 2022; Botezatu *et al.,* 2014). In adult cattle, erythrocytes are 5 to 6 µm broad, show low core pallor, and have a very short lifespan of around 130 to 150 days (Adili and Melizi, 2014). However, these erythrocytes may also exhibit cytological abnormalities in form, size, coloration, and even intra-erythrocyte inclusions (Derkho *et al,* 2019). These abnormalities can be detected in a blood smear under a microscope, where shape abnormalities can be distinguished by identifying the cell shape, as they can be spherocytes, elliptocytes, schistocytes, acanthocytes, echinocytes, keratocytes, codocytes (target cells), stomatocytes, dacryocytes (drop cells), and sickle cells, however, the presence of two or three shapes indicates poikilocytes (Derkho *et al,* 2019). Size anomalies, determining the cell size, as they can be microcytes, macrocytes, or anisocytes, the latter signifying the existence of both sizes at the same time (Adewoyin *et al.,* 2019). Regarding erythrocyte staining anomalies, hypochromia, polychromatophilia (strong staining), and anisochromia (unequal staining of red blood cells) can be observed in blood smears (Adewoyin *et al.,* 2019). Regarding intra-erythrocytic inclusion abnormalities detected under the microscope in bovine blood smears, identifying the existence of inclusions within the erythrocytes such as Howell-Jolly bodies (Drieu, 2009), basophilic stippling, and internal infections such as Babesia spp. (Adjou *et al.,* 2015a, b). These inclusions can signal medullary regeneration, a parasite infection, oxidative stress, or a medullary lesion (Fakoya and Amraei, 2024; Herman *et al.,* 2023; Rothmann *et al.,* 1998). To our knowledge, no study has yet been done in Algeria to explore the presence of these erythrocytic abnormalities in animals, notably dairy cows. Therefore, the purpose of this study was to identify numerous erythrocyte anomalies, including their form, size, color, and the existence of intra-erythrocytic inclusions, and to determine their prevalence according to age.

Materials and Methods

This study was conducted on 50 local crossbred dairy cows from January to May 2025 in Tiaret, in western Algeria. The climate of this region is semi-arid, with harsh winters (cold and humid) and hot, dry summers, with average seasonal temperatures of 6°C in winter and 25.9°C in summer (Hallouz *et al.,* 2018). The annual rainfall is irregular, with a maximum of 450 mm in the northern zone and generally <300 mm in the southern zone of Tiaret (Benahmed *et al.,* 2016). All the cows underwent a general clinical examination before the blood sample collection to ensure they were free from any disease. All relevant data, such as age, gestational status, and parity, were recorded for each cow. Regarding feeding, each cow received an average of 9 kg of corn and hay concentrate, and access to water was ad libitum. The cows were divided into age groups viz ≤ 3 years, 3 to 6 years, and o> 6 years.

All blood samples were taken early in the morning via venipuncture (jugular vein) and deposited directly into a tube containing the anticoagulant EDTA.The samples were transferred directly to the laboratory to perform an erythrocyte lineage hemogram using the Orphy Methic 18 hematology analyzer and to prepare blood FTa smears stained using the MGG staining technique (El Bekkali, 2016; Piaton *et al.* 2015). All smears were read under a binocular optical microscope of the Optika brand at a magnification of 1,000x, and 2000 red blood cells, were viewed. The number of red blood cells and the type of abnormality present were recorded, expressed as a percentage of red blood cells. Erythrocyte abnormalities, such as shape, size, and colour, as well as the presence of inclusions, were then graded according to their presence: rare (0.05 to 0.5%), mild (>0.5 to 3%), moderate (>3 to 10%), or pronounced (>10%) (Bajrić *et al.,* 2020; Christopher *et al.,* 2014; Harvey, 2011). Microscopic examination of a single blood sample can reveal multiple anomalies simultaneously. Since various

Table 1. Erythrocyte parameters of the hemogram in the studied cattle

Hemogram (Erythrocyte line)	N	Mean and standard deviation	Reference range
RBCx10^6 /mm^3	50	07.02 ± 1.00	[5- 10][a], [7,6][b], [5,82±0,91][c]
Haemoglobin (Hb; g/dl)	50	10.47 ± 1.31	[8- 15][a], [9,34±1,28][c]
Haematocrit Hct (%)	50	33.41 ± 4.69	[24- 46][a] , [26,73±3,93][c]
Mean Corpuscular Volume (MCV; fL)	50	47.72 ± 5.98	[40-80][a] , [46,25±4,23][c]
Mean Corpuscular Haemoglobin (MCH; pg)	50	14.65± 2.67	[11- 17][a], [16,17±1,48][c]
Mean Corpuscular Haemoglobin Concentration (MCHC; g/dl)	50	31.37 ± 1.02	[30- 36][a] , [35,03±1,59][c]

[a]Kahn and Line, 2005; [b]Cordonnier and Fontaine, 2001; [c]Hariche, 2021.

abnormalities—such as those affecting shape, size, color, or the presence of inclusions—can coexist within the same sample, it is possible to observe multiple types of anomalies in a single smear. Therefore, the total number of abnormalities identified is not dependent on the number of blood smears analyzed, but rather on how many times each specific abnormality occurs across all examined smears.

The whole blood count values were analysed using IBM SPSS 27 software to determine the mean and standard deviation of the red blood cell count. The blood smear results were semi-quantitative, determining the amount and percentage of red blood cells having erythrocyte abnormalities.

Results and Discussion

The results of the hemogram are presented in Table 1. The amount of red blood cells, the level of haemoglobin, the hematocrit, the MCV (Mean Corpuscular Volume), the MCH (Mean Corpuscular Haemoglobin), and the MCHC (Mean Corpuscular Haemoglobin Concentration) are all important signs of haematological state and overall health. Age and parity are two physiological factors that affect these parameters in cattle (Hariche, 2021). The results of the current investigation show that the average number of red blood cells is 7.02±1.00x10^6 mm^{-3}. This number is in the range set by Kahn and Line (2005), which is 5 - 10 x 10^6 mm^{-3}, and it is near to the average recorded by Cordonnier and Fontaine (2001), which is 7.6 x 10^6 mm^{-3}. This average number, on the other hand, is still larger than the one Hariche (2021) obtained, which was 5.82±0.91 x 10^6 mm^{-3}. The normal level of haemoglobin (Hb) is 10.47±1.31 gdl^{-1}. Kahn and Line (2005) say that this figure is within the reference range of 8–15 gdl^{-1}. Hariche (2021) says that the average is 9.34±1.28 gdl^{-1}, which is higher than this amount. Similarly, the average value of hematocrit (Ht) is 33.41±4.69%, which likewise remains within the standard range (24 to 46%) suggested by Kahn and Line (2005), but it is higher than that reported by Hariche (2021), which is 26.73 ± 3.93%. The table shows that the average value of the mean corpuscular volume (MCV) for the Wintrobe erythrocyte indices is 47.72±5.98 fL. According to Kahn and Line (2005), this figure is within the reference range of 40 to 80 fL. It is also similar to the value given by Hariche (2021), which is 46.25 ± 4.23 fL. The mean corpuscular hemoglobin is 14.65 ± 2.67 pg, which is still within the normal range (11-17 pg) (Kahn and Line, 2005), although it is lower than the average found by Hariche (2021), which is 16.17 ± 1.48 pg. As for the mean corpuscular hemoglobin concentration (MCHC), it stands at 31.37 ± 1.02 gdl^{-1}, falling within the normal limits (30-36 gdl^{-1}) according to Kahn and Line (2005), but staying lower than that reported by Hariche (2021), which is 35.03 ± 1.59 gdl^{-1}. The results obtained in the framework of our investigation show that the studied cows presented an overall normal hemogram, without noticeable aberrations in the quantitative parameters. According to Bellier and Cordonnier (2010), a blood count within normal ranges is indicative of a healthy animal.

Of the 50 blood samples analysed, 34 (68%) showed abnormal red blood cells, while the remaining 16 (32%) showed normal red blood cells (no red blood cell abnormalities. These results clearly demonstrate the prevalence of cellular abnormalities in the cows under study. Our results compare with those of Chaudhry *et al.* (2018), who found 260 cases of red blood cell abnormalities out of a total of 280 cases, representing 92.85% of the total blood samples examined. However, our results differ from those of Rehman *et al.* (2024), who recorded

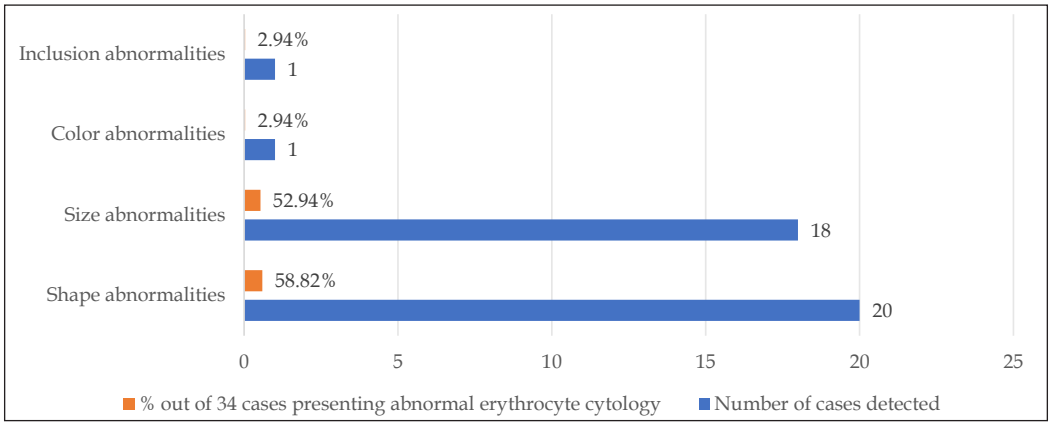

Fig. 1. Frequency of types of erythrocyte abnormalities (alone or in combination on the blood smear).

abnormal cells in only 33.1% of blood samples. According to Watermill (2017), cows suffering from clinical diseases or nutritional deficiencies typically exhibit red blood cell abnormalities not found under normal physiological conditions.

Regarding the general types of abnormalities observed in the cases studied and the distribution of red blood cells exhibiting these irregularities, the combined analysis of data from Figures 1 and 2 indicates a clear predominance of shape abnormalities, followed by size abnormalities. However, the distribution of these abnormalities varies across different blood smears. Shape abnormalities were recorded in 20 out of the 34 cases presenting red blood cell abnormalities, constituting 58.82% of the detected abnormalities, either alone or in combination with other abnomalities. After examining the presence of these abnormalities in each blood smear, the results showed that only 25% of the cases exhibited a high prevalence of red blood cells with shape abnormalities, while 20% showed a moderate prevalence. The remaining cases were either mild (35%) or rare (20%).

In terms of size abnormalities, were recorded in 18 cases, or 52.94% of the observed abnormalities, whether they appeared alone or in association. Blood samples examined under a microscope revealed an uneven distribution of red blood cells with abnormalities sizes. These abnormalities were pronounced (rate of 38.89%) and moderate (rate of 33.33%), while their mild or uncommon presence did not surpass 16.67% and 11.11%, respectively. On the contrary, color abnormalities and inclusion anomalies remained exceptional, each being detected in only one case (2.94%), and the microscopic examination of these two cases revealed a pronounced presence of color abnormalities and a slight presence of inclusion abnormalities. Our results are similar to those of Rehman *et al.* (2024) who reported that shape abnormalities are predominant in cattle, with an average value of 24.7±0.2, followed by size abnormalities, with an average value of 8.4±0.1. On the other hand, our results are in disagreement with those reported by Chowdary *et al.* (2018), who indicated that size

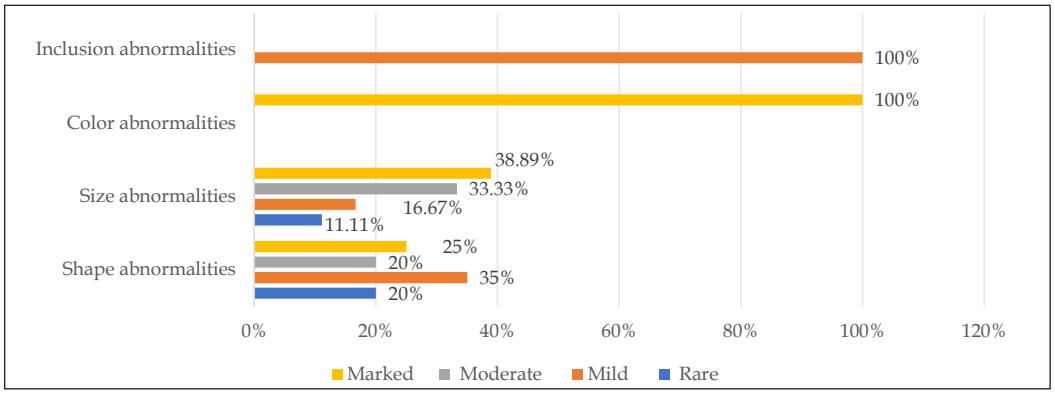

Fig. 2. Semi-quantitative results of each type of erythrocyte abnormalities (alone or in combination on the blood smear).

Table 2. Blood smear results (different types of red blood cell abnormalities)

Type of abnormalities	Detected cells	Number of cases	%
Shape abnormalities (% out of 20 cases)	Poikilocytes	1	5
	Acanthocytes	10	50
	Dacryocytes	6	30
	Rouleaux formation	1	5
	Echinocytes	2	10
	Other forms	0	0
Size abnormalities (% out of 18 cases)	Anisocytes	16	88.89
	Microcytes	2	11.11
	Macrocytes	0	0
Color abnormalities (% out of 01 case)	Anisochromia	0	0
	Hypochromia	1	100
	Hyperchromatophilia	0	0
Inclusion abnormalities (% out of 01 case)	Howell-Jolly bodies	1	100
	Heinz bodies	0	0
	Basophilic stippling	0	0
	Other inclusion	0	0

abnormalities were the most frequent, followed by shape abnormalities.

From the results recorded in (Table 2), it is clear that among the shape abnormalities, acanthocytes (Fig. 3) are the most common type in blood smears. Dacryocysts (Fig 3) were also observed in six cases, but other types were very rare. According to Derkho *et al.* (2019), The appearance of acanthocytes, resulting from changes in the structure of blood cells, and their functions in the context of toxic effects of elements present in copper and zinc pyrite ore. Regarding size abnormalities, most cases presented anisocytosis; two cases of microcytosis were also observed. This study did not identify any cases of macrocytosis, indicating that this condition is not very common. In all blood smears, only one case of hypochromia was observed, illustrating the rarity of this type of condition in the analyzed sample. The data also show the presence of a single inclusion, called a Howell-Jolly body. This observation supports the idea that this type of abnormality is very rare in the group studied.

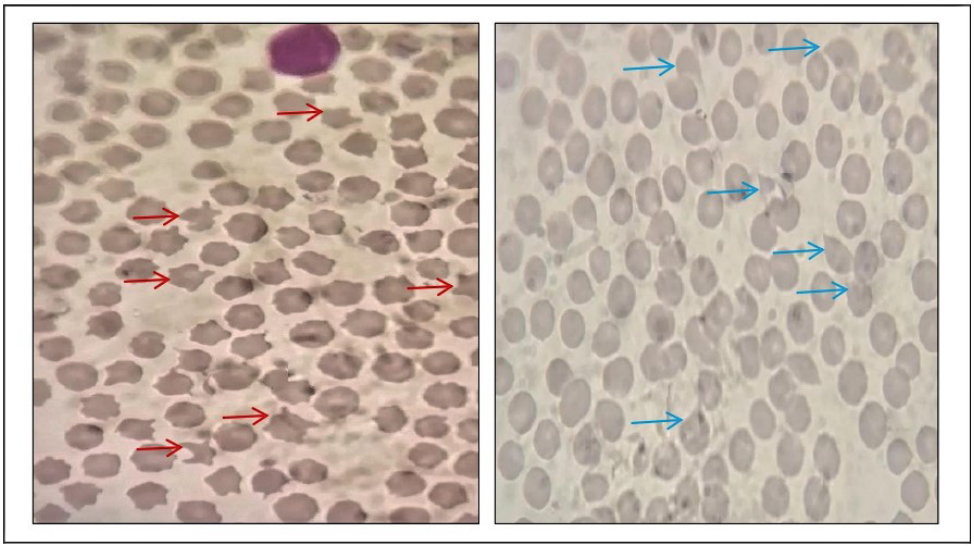

Fig. 3. Some shape abnomalities detected on blood smears of cattle (G×100) (Red arrow for acanthocytes, blue arrow for dacrocytes).

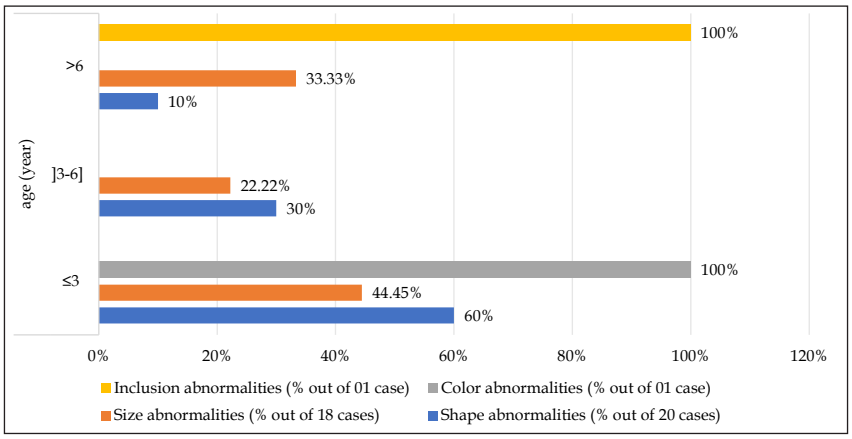

Fig 4. Prevalence of types of erythrocyte abnormalities according to age.

Table 3. Blood smear results (different types of red blood cell abnormalities)

Type of abnormalities	Age and numbre	Detected cells	Number of cases	Per cent
Shape abnormalities (% out of 20 cases)	≤ 3 years (12 cases)	Poikilocytes	1	5%
		Acanthocytes	6	30%
		Dacrocytes	5	25%
		Other forms	0	0%
	[3-6] years (6 cases)	Acanthocytes	4	20%
		Dacrocytes	1	5%
		Rouleaux formation	1	5%
		Other forms	0	0%
	> 6 years (2 cases)	Echinocytes	2	10%
		Other forms	0	0%
Size abnormalities (% out of 18 cases)	≤ 3 years (8 cases)	Anisocytes	7	38.90%
		Microcytes	1	5.55%
		Macrocytes	0	0%
	[3-6] years (4 cases)	Anisocytes	4	22.22%
		Other sizes	0	0%
	> 6 years (6 cases)	Anisocytes	5	27.78%
		Microcytes	1	5.55%
		Macrocytes	0	0%
Color abnormalities (% out of 01 case)	≤ 3 years (1 case)	Anisochromia	0	0%
		Hypochromia	1	100%
		Hyperchromatophilia	0	0%
	[3-6] years (0 cases)	Anisochromia	0	0%
		Hypochromia	0	0%
		Hyperchromatophilia	0	0%
	> 6 years (0 cases)	Anisochromia	0	0%
		Hypochromia	0	0%
		Hyperchromatophilia	0	0%
Inclusion abnormalities (% out of 01 case)	≤ 3 years (1 case)	Howell-Jolly bodies	1	100%
		Other inclusion	0	0%
	[3-6] years (0 cases)	Howell-Jolly bodies	0	0%
		Other inclusion	0	0%
	> 6 years (0 cases)	Howell-Jolly bodies	0	0%
		Other inclusion	0	0%

Prevalence of types of erythrocyte abnormalities according to age

Regarding the age factor, the analysis of the prevalence and distribution of morphological abnormalities in the erythrocytes of the studied cows reveals several trends. (Fig. 4) and (Table 3) show an apparent association between age and the frequency of erythrocyte alterations. Young cows (≤3 years) have the highest proportion of abnormal blood smears. With a continued predominance of shape abnormalities with 12 cases followed by size abnormalities with 6 cases. These shape abnormalities are mainly acanthocytes with 6 cases, while dacryocytes presented 5 cases. However, the low frequency of shape abnormalities was recorded in older cows (>6 years), with only 2 cases of echinocytes. Our results are similar to those of Rehman *et al.* (2024). This distribution of the studied abnormalities indicates that young cattle are particularly sensitive to erythrocyte disorders, which may be related to their physiological or immunological status. Several studies support our recorded observations, most notably Mohri *et al.* (2007), who reported that young cattle often suffer from impaired erythrocyte formation, which may lead to fluctuations in mean corpuscular volume and decreased hematopoietic efficiency. Furthermore, Golbeck *et al.* (2019) explain that the maturation of the hematopoietic system in young animals is not yet fully stabilized, which makes the erythrocyte profile more variable. Indeed, the predominance of shape and size anomalies in young cattle can be explained by their increased sensitivity to nutritional imbalances, iron deficiencies, as well as the physiological stress related to growth. According to Derkho *et al.* (2019); Moroz *et al.* (2012) and Weiss and Wardrop (2011), young cattle are often more exposed to oxidative stress or subclinical parasitic infestations, which can disrupt erythrocyte production. Moreover, the occurrence of size anomalies at this age could also reflect increased regenerative activity of the bone marrow, which is physiologically consistent with a phase of rapid growth. On the other hand, older cattle (>6 years) exhibit fewer anomalies, which could indicate a better erythrocyte stability acquired with age, provided their nutritional and health status is satisfactory.

Conclusions

The above study leads to the conclusion that red blood cell abnormalities are present in cattle, but at varying rates, ranging from rare to moderate, mild, and severe. These abnormalities are more common in young cattle and very rare in older cattle. Shape abnormalities, particularly acanthocytes, are the most common, while size abnormalities, such as anisocytes, are very common in cows. On the other hand, Color abnormalities and inclusions, appear to be very rare.

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