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Leukocyte Subpopulations in the Peripheral Blood of the Omani Camel Breed

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ABSTRACT

Breed-specific variations in immune responses have been studied across various species and breeds. The identification of camel breeds with high immune competence can enhance the breeding of camels with superior immune responsiveness. To date, no study has examined the immune cell composition in the blood of the Omani camel breed. The present study aimed to analyze the immunophenotype of blood leukocytes in the Omani camel breed and investigate the impact of age and gender on the tested immune parameters. To do so, blood samples were collected from 32 clinically healthy camels, randomly selected and comprising 17 camel calves (8 males and 9 females) and 15 adult camels (4 males and 11 females). The samples were tested using flow cytometry and membrane immune fluorescence. The results of the present study revealed a significantly lower count of white blood cells (WBC) in the Omani camel breed than the reference ranges reported for dromedary camels in the published literature. The leukogram was characterized by the dominance of neutrophils (54-60 %) in the blood, followed by lymphocytes (23-33 %). When compared to adult camels, the leukogram pattern in young Omani camels was characterized by elevated WBC and lymphocytes but low levels of eosinophilic granulocytes. The analysis of lymphocyte subsets revealed the dominance of γδ T cells over helper T cells and B cells in the blood of young camel calves, confirming that camels belong to the γδ T cell-rich species. In addition, lower numbers of B cells and helper T cells in young camels suggest lower cell-mediated and humoral immune functionality compared to adults. Although some differences were identified between male and female adult camels, these results are limited by the low numbers of male camels within the adult group. In conclusion, the distinct leukogram patterns observed in young and adult camels highlight the significant impact of age on the immune competence of Omani camels.

Keywords: Camel, Omani camel, Immunophenotype, Leukocyte

INTRODUCTION

The dromedary camel (*Camelus dromedarius*) is well-adapted to the desert, with the ability to grow, reproduce, and produce milk under harsh environments. As far as scientific research is concerned, camels have lagged behind other livestock species for a long time. However, in recent years, research on camels has gradually gained increased interest (Hoter et al., 2019; Tibary and El Allali, 2020).

Within the dromedary camel species, several breeds have been characterized based on morphological characteristics, geographical distribution, and genomic structure (Alhaddad and Alhajeri, 2019). Mainly characterized camel breeds include Al-Mujaheem, Al-Mugateer, Shaele, Homor, and the Omani camel breed (Alhaddad and Alhajeri, 2019; Almathen et al., 2022). The Omani camel breed, primarily found in the eastern regions of Oman, Saudi Arabia, and some regions across the United Arab Emirates is particularly notable as a racing camel population in the Arabian Peninsula (Almathen et al., 2022).

In both human and veterinary medicine, total and differential leukocyte counts, collectively referred to as the leukogram or leukon, have proven to be valuable and cost-effective tools for the evaluation of the physiological and pathological status, and these assessments aid in diagnosis, therapy, and prognosis (Ajadi et al., 2018; Balmant et al., 2018; Braun et al., 2021). In dromedary camels, the main leukocyte populations as well as several lymphocyte subsets - including CD4+ ab T cells, WC1+ γδ T cells, and B cells - have been characterized (Hussen and Schuberth, 2020). Previous studies show that camels, like other species from the artiodactyls group such as cattle and pigs, are classified as γδ-high species, meaning γδ T cells constitute a significant proportion of T cells in the blood of newborns and young animals. This contrasts with γδ-low species like humans and mice, where γδ T cells represent only a minor fraction of blood T cells (Guzman et al., 2014; Hussen and Schuberth, 2020). Previous studies have also explored the influence of several physiological parameters, such as age, gender, and pregnancy on camels' cellular camel immune system (Gaashan et al., 2020; Hussen et al., 2020).

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Despite these efforts, limited data are available on the immune cell composition in the blood of the Omani camel breed. This study was conducted to investigate the immunophenotype of blood leukocytes in Omani camels and evaluate the impact of age and gender on leukocyte distribution patterns.

MATERIALS AND METHODS

Ethical approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of King Faisal University, Saudi Arabia (KFU-REC-2024-JUN-ETHICS1843)

Animals and blood sampling

The study was conducted on an Omani camel breed reared on a private camel farm located in Hofuf City (Eastern Province of Saudi Arabia, Al-Ahsa Region). Blood sampling was performed on October 19, 2024, at 11:00 AM. A total of 32 camels were randomly selected from a population of 55 camels on the farm. The study population included 17 camel calves (aged 5-18 months) and 15 adult camels (aged 3-9 years). The young group consisted of 8 male and 9 female calves, while the adult group comprised 4 male and 11 female camels (Table 1). All adult female camels were nonpregnant and non-lactating at the time of sampling. The camels were kept under a traditional management system, where they grazed on grazing plants such as *Aeluropus lagopoides, Anabasis setifera, Atriplex halimus, Calligonum comosum* during the daytime and were housed in a fen barn at night. Water was provided *ad libitum*. All camels were clinically healthy based on clinical examination performed by a trained veterinarian. Blood samples (5 mL) were collected from the jugular vein using vacutainer tubes containing EDTA (Guangzhou Improve Medical Instruments Co., Ltd., Guangzhou, China) and transported to the laboratory within one hour of collection.

Table 1. Age (year) and gender of the investigated Omani camels sampled in October 2024 in Saudi Arabia

Age	**Calf camel**		**Adult camel**	
	Male	**Female**	**Male**	**Female**
Number	**8**	**9**	**4**	**11**
Minimum	0.5	0.5	3.0	5.0
25% Percentile[1]	1.0	0.5	3.8	5.0
Median	1.0	1.0	6.0	8.0
75% Percentile[2]	1.7	1.3	7.5	10.0
Maximum	1.8	1.5	8.0	10.0
Mean	1.2	0.9	5.8	7.6
Std. Deviation	0.4	0.4	2.1	2.0
Std. Error[3]	0.1	0.1	1.0	0.6

[1]: The value at which 25% of the values lie below that value, [2]: The value at which 75% of the values fall below this value, [3]: Standard error.

Cell separation and flow cytometry

Leukocytes were separated using hypotonic lysis of erythrocytes followed by centrifugation, as previously described by Alhafiz et al. in 2022. Briefly, 1 mL of EDTA-treated blood was incubated with 5 mL distilled water in a 15 mL sterile falcon tube for 20 seconds to induce red blood cell lysis. Subsequently, tonicity was restored by the addition of 5 mL of 2x phosphate buffered saline (PBS), and the tube was centrifuged at 3000 RPM for 10 min at 10ºC. The lysis step was repeated twice with centrifugation at 2200 and 1500 RPM for 10 min until complete erythrolysis was achieved. Finally, the cells were suspended in cold PBS (1×10^6 cells / mL). Cell vitality was measured by the addition of propidium iodide, consistently exceeding 90% (Alhafiz et al., 2022). Total white blood cell (WBC) counts were determined using a Neubauer cell counter and light microscopy after diluting blood samples with Türk's solution (1:10). The absolute cell count of each cell subset was calculated by multiplying cell percentage by the total WBC count.

Flow cytometric analysis of leukocyte subsets

Monoclonal antibodies (mAbs) specific to cell markers BAQ44A (B cell), WC1 (γδ T cell), and CD4 (helper T cell) were used for cell labeling (Hussen et al., 2023). Cells were first incubated in a 96-well plate (1×10^5 cells / well) with the primary mAbs for 15 min at 4°C. After washing with PBS/BSA buffer, the second staining step was done by adding fluorochrome-labeled antibodies (10 µL of each mAb diluted 1: 100 in PBS/BSA buffer) specific to mouse IgM, IgG1, and IgG2a (Invitrogen) followed by incubation for 15 min at 4°C. Following a final wash, the cells were analyzed using an Accuri C6 flow cytometer (Becton Dickinson Biosciences, San Jose, California, USA).

Statistical analyses

Mean values, standard deviation (SD), and standard error of the mean (SEM) were calculated using the column statistics function in the Prism software (GraphPad). Data normality was assessed using the Shapiro-Wilk test. Comparisons between means were performed using paired student's t-test for normally distributed data or Mann-Whitney test for non-normally distributed data, with p values less than 0.05 indicating significant effects.

RESULTS AND DISCUSSION

Relative percentages and absolute counts of leukocyte subpopulations

Omani camel calves showed significantly ($p < 0.05$) lower percentages of neutrophils (54.9%) and eosinophils (3.2%) compared to adult camels (60.7% for neutrophils and 7.8% for eosinophils) ($p < 0.05$). In contrast, the percentage of lymphocytes was significantly higher in young camels (33.3 %) than in adults (23.9%; $p < 0.05$). The percentage of monocytes did not differ significantly between the two age groups ($p > 0.05$). Compared to adult camels, camel calves showed significantly higher numbers of total leukocytes (6176 versus 4753 cells/µL blood in adult camels) and lymphocytes (2076 versus 1120 cells/µL blood in adult camels), but lower numbers of eosinophils (204 versus 360 cells in adult camels) ($p < 0.05$). No significant differences were observed between the two groups regarding the absolute numbers of neutrophils and monocytes ($p > 0.05$) (Figure 1A-B).

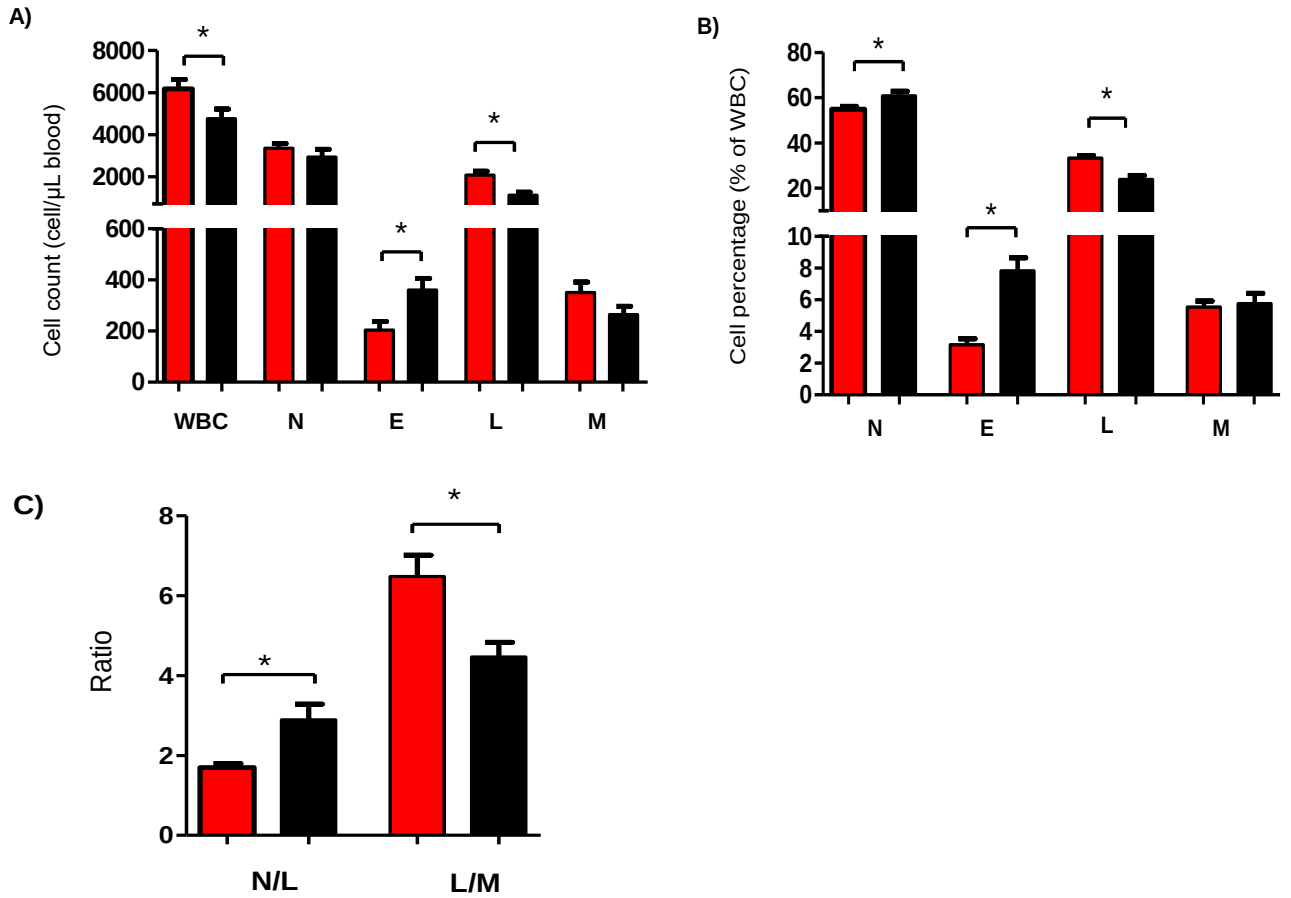

Figure 1. Leukogram patterns in young (red color) and adult (black color) Omani camels. **A**: Absolute count of neutrophils (N), eosinophils (E), lymphocytes (L), and monocytes (M) as quantified by flow cytometry. **B**: Relative percentages of total white blood cells (WBC) and the different leukocyte subpopulations in young and adult Omani camels. **C**: The neutrophil-to-lymphocyte (N/L) and lymphocytes-to-monocytes (L/M) ratios in young and adult Omani camels. *: $p < 0.05$.

Neutrophils to lymphocyte and lymphocyte to monocyte ratios

The neutrophils-to-lymphocyte ratio (N/L) was significantly ($p < 0.05$) lower in young camels (1.7) than in adult camels (2.9), while the lymphocyte-to-monocyte ratio (L/M) was significantly higher in the young camel group (6.5) than in the adults (4.5) ($p < 0.05$, Figure 1C).

Lymphocyte subsets in camel blood

The analysis of camel lymphocyte subsets revealed a significantly ($p < 0.05$) higher percentage of WC1+ T cells within total lymphocytes in young camels (23.4% of lymphocytes) compared to adult camels (7.4%). Conversely, the percentages of CD4 + T cells (13.3 versus 18.6% in adult camels) and B cells (8.4 versus 17.0% in adult camels) were significantly ($p < 0.05$) lower in camel calves than in adult camels (Figure 2A).

Absolute counts of lymphocyte subsets showed only significantly ($p < 0.05$) higher numbers of WC1+ T cells in young (476 cells/µL blood) than in adult (88 cells/µL) camels. However, no significant differences were observed in the absolute counts of CD4+ T cells and B cells between the two age groups ($p > 0.05$, Figure 2B).

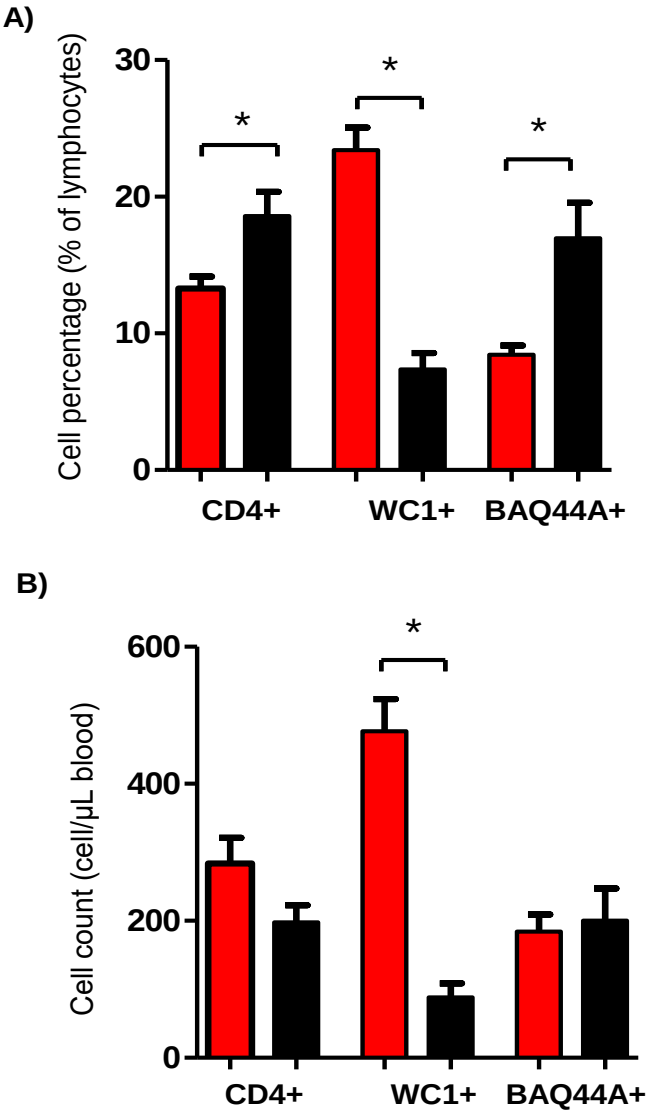

Figure 2. Lymphocyte subsets in young (red color) and adult (black color) Omani camels. **A:** Relative percentages of CD4 + T cells, WC1 + T cells, and BAQ44A+ cells (B cells) as quantified by flow cytometry. **B:** Absolute counts of CD4 + T cells, WC1 + T cells, and B cells in young (red color) and adult (black color) Omani camels. *: $p < 0.05$.

Impact of gender on the immune cell composition

The distribution of leukocyte subpopulations, their absolute counts, and the ratios in male and female camels are shown in Tables 2, 3, and 4. Comparisons between males and females within the two age groups revealed significant differences in the percentage of lymphocytes, the neutrophils-to-lymphocyte ratio, and the number of eosinophils ($p < 0.05$). The percentage of lymphocytes was significantly higher in female (26.1 % of WBC) than in male adult camels (17.5 % of WBC; Table 2). In contrast, the neutrophils-to-lymphocyte ratio was significantly lower in females (2.3) than in (4.3) adult males (Table 2). Male adult camels (525 cell/µL) showed significantly higher ($p < 0.05$) numbers of eosinophils than female adult camels (301 cell/µL; Table 3).

Table 2. Relative percentages of leukocyte subpopulations in blood samples collected from Omani camels (October 2024 in Saudi Arabia)

		Calf camel (5 – 18 months)		Adult camel (3 to 9 years)	
		Male (n =8)	**Female (n = 9)**	**Male (n = 4)**	**Female (n = 11)**
Neutrophils (% of WBC)	Minimum	49.6	47.3	59.2	45.2
	Maximum	62.2	64.2	79.2	66.4
	Mean	54.9	54.8	66.4	58.6
	SD	4.1	6.2	9.0	6.4
Eosinophils (% of WBC)	Minimum	0.8	1.3	6.5	2.8
	Maximum	4.9	6.9	14.6	11.1
	Mean	2.7	3.5	10.0	7.0
	SD	1.3	1.6	3.4	2.8
Lymphocytes (% of WBC)	Minimum	27.9	25.8	10.2	19.3
	Maximum	40.2	41.6	22.4	39.9
	Mean	33.5	33.1	17.5	26.1*
	SD	4.1	5.2	5.3	5.9
Monocytes (% of WBC)	Minimum	3.9	3.5	3.6	3.5
	Maximum	8.5	7.2	5.2	12.4
	Mean	5.8	5.3	4.3	6.2
	SD	1.6	1.4	0.7	2.7
Neutrophils/Lymphocytes	Minimum	1.2	1.1	2.7	1.1
	Maximum	2.2	2.4	7.8	3.4
	Mean	1.6	1.7	4.3	2.3*
	SD	0.3	0.4	2.3	0.6
Lymphocytes/Monocytes	Minimum	4	3.6	2.8	2.1
	Maximum	9.4	10.5	5.2	7.7
	Mean	6.2	6.7	4	4.6
	SD	2.0	2.3	1.1	1.5

*: Indicates significant impact of gender, SD: Standard deviation, n: Number. * indicates a significant difference at $p < 0.05$ in a row.

Table 3. Absolute cell counts of leukocyte subpopulations in blood samples collected from Omani camels (October 2024 in Saudi Arabia)

		Calf camel (5 – 18 months)		Adult camel (3 to 9 years)	
		Male (n =8)	**Female (n = 9)**	**Male (n = 4)**	**Female (n = 11)**
WBC (cell/µL)	Minimum	4000	3700	3500	2500
	Maximum	8800	8500	9100	8000
	Mean	6413	5967	5500	4482
	SD	1854	1913	2551	1543
Neutrophils (cell/µL)	Minimum	2024	1994	2125	1540
	Maximum	4733	4484	7209	4363
	Mean	3503	3216	3798	2609
	SD	943	925	2321	877
Eosinophils (cell/µL)	Minimum	43	47	344	124
	Maximum	356	521	803	487
	Mean	179	227	525	301*
	SD	114	155	216	122
Lymphocytes (cell/µL)	Minimum	1254	1013	665	483
	Maximum	3195	2786	1128	2617
	Mean	2165	1999	876	1209
	SD	739	764	199	616
Monocytes (cell/µL)	Minimum	192	157	167	135
	Maximum	747	598	331	548
	Mean	374	330	229	277
	SD	170	176	71	141

*: Indicates significant impact of gender, SD: Standard deviation, n: Number. * indicates a significant difference at $p < 0.05$ in a row.

Table 4. Lymphocyte subsets in blood samples collected from Omani camels (October 2024 in Saudi Arabia)

		Calf camel (n = 17) (5 – 18 months)		Adult camel (n = 15) (3 to 9 years)	
		M (n =8)	**F (n = 9)**	**M (n = 4)**	**F (n = 11)**
CD4+ T cells (% of lymphocytes)	Minimum	9	7.2	9.1	10.4
	Maximum	18.9	21.5	28	30
	Mean	13.58	12.98	21.6	17.42
	SD	3.17	3.989	8.588	6.236
gd T cells (% of lymphocytes)	Minimum	12.1	15.4	4.1	2.5
	Maximum	30.9	37.1	11.3	18.9
	Mean	20.7	25.82	9	6.773
	SD	6.116	6.764	3.317	4.995
B cells (% of lymphocytes)	Minimum	4.89	4.7	6.33	7.3
	Maximum	12.56	12.12	32.61	38.9
	Mean	8.129	8.714	14.79	17.76
	SD	3.021	2.722	12.2	9.807
Helper T cells (cell/µl)	Minimum	160	113	84	100
	Maximum	603	594	297	433
	Mean	304.4	265.4	187.8	201.4
	SD	161.7	154.8	90.85	102.9
γδ T cell (cell/µl)	Minimum	181	256	27	13
	Maximum	668	826	121	314
	Mean	445.8	504.7	82.25	90.64
	SD	181.6	211.3	39.56	91.14
B cell/µl	Minimum	68	64	59	64
	Maximum	374	335	302	725
	Mean	182.9	185.1	129	225.8
	SD	104	110.2	115.8	199.3

gd: Gamma delta T cells, B cell: B lymphocytes, n: Number, M: Male, F: Female.

Breed-specific differences in the immune response have been studied for several species and breeds (Hadfield et al., 2018; Lawrence et al., 2013). In the present study, the total WBC counts in the Omani camel breed ranged between a minimum value of 2500 and a maximum value of 9100 cells/µL blood (mean ± SD: 5509 ± 1940), which is lower than the reference ranges reported for the dromedary camels in previous studies (Al-Busadah, 2007; Al-Mujalli et al., 2011; Martin-Barrasa et al., 2023). Whether these results represent a characteristic range for the Omani camel breed or not requires further comparative studies with other camel breeds. For instance, Martin-Barrasa et al. (2023) reported total WBC counts for the Canary Island dromedary camels ranging from 7.35 and 18.36 $X10^3$ cells/µL. Similarly, Fey and Bengoumi (2018) reported WBC values between 9.7 and 20.1 $X10^3$ cells/µL. The dominance of neutrophils (58 ± 7 % of WBC) followed by lymphocytes (29 ± 7 % of WBC) in blood aligns with previous literature on dromedary camels (Zongping, 2003; Vap and Bohn, 2015).

The influence of age on the immune system has been addressed in several previous studies (Romanyukha and Yashin, 2003; Elghetany and Lacombe, 2004). In a recent study, Martin-Barrasa et al. (2023) compared leukograms between young and adult dromedary camels in the Canarian Islands and reported higher total WBC counts, along with higher neutrophils, lymphocytes, and monocytes counts, in calves compared to adults. A similar leukogram pattern was also observed in camel calves in Saudi Arabia (Gaashan et al., 2020). The results of the present study corroborate with the literature reports regarding the elevated numbers of WBC and lymphocytes and lower numbers of eosinophils in camel calves (Gaashan et al., 2020). However, while previous studies reported higher frequencies of neutrophils and monocytes in young than in adult camels (Martin-Barrasa et al., 2023), the present study found comparable numbers of neutrophils and monocytes between young and adult Omani camels. Whether this observation is specific to the Omani camel breed requires further investigation, including comparisons with other camel breeds.

Newborn and young artiodactyls, including camels, cattle, goats, sheep, and pigs, are characterized by high percentages of γδ T cells in their blood. In newborn camel calves, γδ T cells account for up to 35 % of blood lymphocytes (Hussen and Schuberth, 2020). In the present study, the dominance of γδ T cells over helper T cells and B cells is in agreement with previous studies. In the present study, the lower numbers of B cells and helper T cells, which are known for their central

role in humoral and cell-mediated immune responses, respectively, further confirm the reported difference in immune functionality between young and adult camels (Hussen and Schuberth, 2020).

CONCLUSION

The present study identified reference values for some immune cell populations in blood from the Omani camel breed. The results indicated the significant impact of age on the analyzed parameters. Specifically, the different frequencies of B cells and T cells suggest lower immune competence in terms of both humoral and cell-mediated immune responses in young camels as opposed to adult camels. Further studies on camels from other breeds could focus on functional differences in the immune system between different camel breeds. Although some differences were observed between male and female adult camels, these findings are limited by the smaller number of male camels in the adult group (n = 4). Therefore, future studies with larger sample sizes are needed to investigate gender-related differences in immune cell composition.

DECLARATIONS

Authors' contributions

Jamal Hussen designed the study, analyzed the data, and wrote the first draft of the manuscript. Fathi Ahmed AL-Musallam collected the samples, counted cells, and revised the manuscript. All authors read and approved the final version of the manuscript.

Ethical considerations

All authors have been screened for ethical issues, including plagiarism, consent for publication, misconduct, fabrication of data, and duplicate publication or submission.

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Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Availability of data and materials

The datasets generated during the current study are available from the corresponding author upon reasonable request.

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