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Hematological reference intervals in newborn dromedary calves in the first week after birth: Age and sex-related variations

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ABSTRACT

The establishment of hematological reference intervals (RIs) is an important tool to assess the health status of animals and to evaluate the impact of diseases at individual and population levels. Nowadays, specific RIs of hematological parameters in newborn dromedary camel calves at birth and during the first week after birth, are lacking. Therefore, RIs for the hematological variables from a complete blood cell count were established in 47 healthy newborn dromedary calves (18 females and 29 males). Blood samples were collected within 2 h after birth (d0), at 24 h (d1), at 3 (d3) and 7 days (d7) after birth, and analyzed within 24 h. The RIs were described based on the 95% confidence interval, and possible differences among mean values due to age (sampling time) and sex were investigated. Statistical analysis showed that age affected all the hematological variables except MCV, MCH, and MCHC, indicating that the adaptational process to the extrauterine life continues for several days after birth; sex affected most of the hematological variables, with higher RBC and PLT count, HGB, PCV, neutrophil population and neutrophil:lymphocyte ratio at d7 in females compared to males. These findings suggest possible sex-based differences in the physiological maturation mechanisms and deserves further investigations. To the best of the authors' knowledge, this is the first report of hematological RIs for newborn dromedary calves at birth up to 7 days of age; the RIs registered in the present study in newborns differ from those reported in adult dromedaries in literature, thus confirming the need for the adoption of separated reference ranges according to age also in the dromedary camel, as previously reported for other species.

1. Introduction

The *Camelus dromedarius,* or one-humped camel, is a domestic species distributed in Asia and Africa, from East India to Morocco, in Canary Islands (Spain), and Central Australia. Because of its adaptation to desert climates and to constraining conditions, this species had an important role in human life history of these regions; nowadays it is still employed as a mean of transportation by local populations, and it provides a substantial contribution in protein supplying through production of meat and of an exceptional nutritive milk (Faye et al., 2014), enabling economic security to locals especially in the context of climate change.

As a matter of fact, in the near future, climate changes will likely decrease agricultural areas for animal production worldwide; in such conditions, extensive animal production could be limited to some extent to semi-arid pastures, severely affecting conventional livestock production, and old-world camelids may become an important protein source for humans in the tropics and subtropics. Notwithstanding the growing potential and opportunities of rearing dromedary camels, this species has not been considered a major player in the international animal production context, until recently. During the last 30 to 40 years, two important changes in the camelid breeding were observed, namely the emergence of more intensive farming and milk processing systems (Faye et al., 2014), and the geographical expansion of this species even into completely new ecosystems. After recommendation by the Food and Agriculture Organization (FAO) of the United Nations (UN), the UN General Assembly has designated 2024 as the *International Year of Camelids*, to raise public awareness about the importance of camelids for food security and ecosystem functions.

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Nowadays, a major cause of production loss in camelids is represented by neonatal mortality (Tibary et al., 2008); although traditionally regarded as an indicator of management quality, neonatal mortality is recently considered a crucial animal welfare indicator, becoming the most commonly used population-level welfare index in dairy cow farms (Mee, 2013). In this context, there is still insufficient information regarding camelids; Bravo et al. (2009) reported a 6% mortality rate until the first week of life in alpacas, while recently Nagy et al. (2023) reported a 3.84% of perinatal mortality (mortality within 48 h after birth) in dromedary camels born in a large, intensive, and well managed camel dairy farm.

The importance of evaluation and primary care for the newborn is essential for the reduction of neonatal mortality and brings up the need for the determination of species-specific blood reference values for the accurate diagnosis of disorders, for measuring disease progression and response to treatment, and for preventative monitoring of individual and herd health. In this regard, the complete blood cell count (CBC) is usually the first line of diagnostics, from which much useful information can be obtained; moreover, the low costs of analysis and the broad availability of automated cell counters have made it more and more popular in veterinary practice (Roland et al., 2014).

The normal ranges, better known as reference intervals (RIs), are derived from a healthy group of individuals, and can be affected by age, sex, genetics, nutritional status, pregnancy, and other multiple factors (Roland et al., 2014). Recently, Faye and Bengoumi (2018) comprehensively summarized the knowledge currently available on camel hematology and conflicting results emerged about possible age or sex-related variations; additionally, when young individuals were considered, population groups consisted of calves of very different ages. Reference values for hematological variables were very recently reported in the Canary camel breed by Martín-Barrasa et al. (2023), but young camels were generally defined as those <1 year old. A focus on dromedary hematology during the first days of life was published by Elkhair and Elmgboul (2015), but the population size was relatively small to obtain RIs, thus generating mean and standard deviation values that include 68% of the population. To the best of the authors' knowledge, the comprehension of physiological changes of hematological parameters in dromedary camel calves during the first week after birth is still lacking, although it is exactly during this time period that most of the adaptational changes take place. Given that the knowledge of RIs is a compelling tool for monitoring animals' health and understanding the impact of disease at individual and population levels (Geffré et al., 2009; Friedrichs et al., 2012), the present study aimed to establish the hematological RIs in healthy dromedary calves at birth and during the first week after birth and to investigate possible differences related to age and sex.

2. Materials and methods

The study was performed in compliance with welfare guidelines and with the regulations for the use of animals for research purposes. The experimental protocol was approved by the ethic committee of the Department of Veterinary Medicine, University of Bari Aldo Moro (approval number 15/2022).

2.1. Sample population and procedures

In March 2023, a group of dromedary camel calves born in a semi-intensive breeding system farm located in Al-Qassim region (Saudi Arabia) was enrolled in the study. The farm was selected because i) RIs should be determined close to the target patient as possible and the semi-intensive breeding system represented the most widespread breeding system in Saudi Arabia; ii) the animals' management was considered optimal, particularly in terms of health and nutrition (i.e. brucellosis and trypanosomiasis tests plus regular external and internal anti-parasites treatments); iii) high number of expected deliveries in a short time and thus in climatic/environmental standard conditions; iv) willingness of the owner to help in the acquisition of new knowledge; v) adequate human resources for monitoring the deliveries and herd care, as well as for helping during the data collection phase. About one hundred female dromedary camels were reared on the farm, and distributed into pregnant and calf-rearing dams. The paddocks were provided with shaded areas, and pregnant animals were left free to graze on pastures for three hours/day, receiving alfa-alfa upon their return. Calf-rearing animals received alfa-alfa and concentrates, while for both groups water was provided at libitum. Parturitions occurred spontaneously, at any time of the day, and were monitored by trained staff to identify abnormalities requiring veterinary intervention. Neonatal health and maturity status was performed by veterinarians soon after birth, according to what reported by Tibary and Anouassi (2021); particularly, prematurity was evidenced by firm attachment of the epidermal membrane to the feet pads, and by the absence of erupted teeth. Health status examinations included: heart rate (normal if 80–120 beats per minute, regular rhythm), respiratory rate (normal if 20–30 breaths per minute, regular rhythm), body temperature (37.5–39 °C), presence of suckling reflex (within the first half-hour after birth). Health status was checked again daily through a complete examination: body temperature, hydration status, calf demeanor, mobility and reactivity, presence of suckle reflex and fecal consistency were evaluated. As per management practices established for the herd, colostrum intake occurred through spontaneous suckling; in case of failure of spontaneous intake within 3 h after birth, colostrum was milked from the dam and administered by bottle.

The exclusion criteria were:

- abnormal gestation length (to avoid inclusion of premature or dismature calves);
- dystocia or any abnormality during and after delivery (to avoid influences of abnormal parturition of the newborn physiological characteristic, e.g. oxygenation);
- alterations of any of the examined physical parameters in newborns (particularly about demeanor and scour), or need of medical treatments (to avoid inclusion of blood samples possibly altered by diseases or treatments).

During the study period, dromedary newborn calves were kept in large paddocks together with their mothers, allowing spontaneous suckling and behavioral interactions.

Each newborn dromedary calf was blood sampled within 2 h (hrs) after birth (d0), and at 24 h (d1), and at 3 (d3) and 7 days (d7) after birth; blood sampling were all performed by the same team, consisting of two veterinarians. The blood samples were collected from the jugular vein into 5 ml K3-EDTA plastic tubes (AFCO©, Amman, Jordan) and stored at 4 °C until analysis. Blood smears were immediately prepared in order to prevent cellular morphological alterations, while the automated cell count was performed within 24 h from collection. To avoid pre-analytical errors, samples with the presence of visible clots were excluded from the study.

2.2. Laboratory analysis

Laboratory tests were performed within 24 h from collection at the Veterinary Camel Hospital of the *Salam Veterinary Group* (Saudi Arabia), situated 30 km from the dromedary camel farm.

Anticoagulated blood was used for a complete blood cell count (CBC) using an automated hematology analyzer (*ABX MICROS ES 60®*, HORIBA, Japan) with its associated tri-levels quality controls (*ABX Minotrol 16* - HORIBA, Japan) and calibrator (*ABX Minocal,* HORIBA, Japan). Software was specifically tailored for camels. Hematologic analyses included: hemoglobin concentration (HGB), packed cell volume (PCV), red blood cell (RBC) count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), and white blood cell (WBC) count with subpopulations differential count (lymphocytes, monocytes,

neutrophils and eosinophils). In addition, the WBC differential count was carried out by manual examination of a blood smear, always performed by the same technician and counting 100 nucleated cells.

2.3. Establishment of RIs

Specific RIs were generated for all sampling times (d0, d1, d3, d7) using a Microsoft Excel spreadsheet with the Reference Value Advisor (v.2.1) set of macroinstructions (Geffré et al., 2011). The software is a set of visual basic macros that performs the following computations, recommended by the International Federation of Clinical Chemistry-Clinical and Laboratory Standards Institute (Horowitz, 2008): descriptive statistics (mean, median, SD, minimum, and maximum values); tests of normality (Anderson–Darling with histograms and Q–Q plots and Box–Cox transformation); RIs generation; outlier analysis. RIs are calculated using standard and robust methods on both non-transformed and transformed data (Box-Cox transformation). The software indicates the best method to define the RIs based on data distribution. If untransformed data have a Gaussian distribution, the 95% reference intervals are calculated using the parametric method. Non-Gaussian data are Box-Cox transformed, and the robust method is used to calculate the RIs if data are also symmetrical. If data are non-Gaussian and symmetrical after transformation, then RIs are calculated using the non-parametric method. A non-parametric bootstrap method is used to calculate the 90% confidence interval for lower and upper limits. Outliers are tested using Dixon–Reed and Tukey's tests; the former detects a single potential outlier based on the ratio of its distance to the nearest value divided by the whole range of values, while the latter is based on the median and interquartile range. Outliers classified as 'suspected' are retained, as recommended by the ASVCP guidelines (Friedrichs et al., 2012), while far outliers are removed from the analysis.

2.4. Statistical analysis

Mean values (± standard deviation, SD) for each hematological parameter were calculated for all the sampling times, and statistical analysis was applied to determine differences for each variable according to age (sampling times) and sex (females vs males); specifically, a multivariate ANOVA with time and sex as fixed factors was applied, followed by a Tukey test. Statistical analysis was performed using Jasp Team (Jasp ver. 0.18.1, 2023). Significance was set for $p < 0.05$.

3. Results

A total of 53 pregnant dams were surveilled. Among these, 3 parturitions required a veterinary intervention, while 3 newborns were affected by alterations of health status demanding for medical treatment at birth or during the first week after birth. Therefore, after the adoption of inclusion and exclusion criteria, a total of 47 newborn dromedary calf were eventually enrolled, among which there were 18 females and 29 males. All these calves were born mature and healthy, after a normal gestation length (354–407 days reported for dromedary camels in Saudi Arabia) (Almutairi et al., 2010) and through a spontaneous parturition without complications, and remained alive and healthy during the study-period.

Median, min-max values, and RIs (with 90% CI for lower and upper limits) for the hematological variables in the 47 newborn dromedary calves are presented in Table 1 and in Fig. 1. Details on the descriptive statistics (mean ± SD) for all the hematological variables according to age and sex are reported in Table 2 and in Fig. 2.

Statistical analysis detected many significant differences according to sampling time and sex in all the hematological variables except for MCV, MCH and MCHC. Sampling time affected results regarding RBC, HGB, PCV and PLT count; all these variables were also affected by sex. No significant differences were found for the erythrocyte indexes (MCV, MCH and MCHC) neither according to sampling time nor sex.

The WBC count, neutrophil and eosinophil count and the neutrophil: lymphocyte ratio were affected by age and by sex. Differences were also found for lymphocyte count and monocyte count but only according to sampling time.

4. Discussion

Results from this study provide the first RIs for hematological variables in newborn dromedary calves at birth and during the first week of life. While numerous references on hematological RIs in llamas and alpacas are available from the literature, only a few reports on dromedary camel could be found, with most of them showing mean values, and all referring to adults or young >1 month. To the best of our knowledge, the only RIs for dromedary species were very recently calculated by Martín-Barrasa et al. (2023) on more than one hundred individuals both young (defined as <1 year old) and adult (>4 years old); this latter study, represents the most suitable study for discussing RIs arising from the present research and therefore visual comparisons were included in the figures. Compared to the RBC RIs reported by Martín-Barrasa et al. (2023), the present RBC RIs tended to be lower, especially in the first days after birth. Elkhair and Elmgboul (2015) reported an RBC range (calculated as mean ± SD × 1.96) very similar to those found in the present study in all sampling times. The mean RBC values found in this study during the first week of age are included in the RBC mean values previously reported in the literature for adult camels, which vary between 6 and 10 × 10^6/mm^3 with extreme values between 5 × 10^6/mm^3 (Chartier et al., 1986) and 12.5 × 10^6/mm^3 (Sharma et al., 1973), thus highlighting a high variability of this parameter. From a general point of view, the high RBC variability observed in camels is a phenomenon very similar to what has been also observed in the majority of ruminants (Soliman and Shaker, 1967). The RBC count variations may be correlated to the stress induced by handling during the blood sampling, but the differences reported in literature among different geographical regions also suggest the existence of geographic variations related to climatic differences (Faye and Bengoumi, 2018).

According to the majority of the references, the mean HGB concentration in camels varies between 9.3 and 15.5 g/dl (Faye and Bengoumi, 2018), and the mean HGB values from the present study always fall within the observed ranges. However, the present HGB reference intervals were wider as compared to those found by Martín-Barrasa et al. (2023). Elkhair and Elmgboul (2015) found high mean HGB values at birth (13 g/dl), similar to the 14.12 ± 2.4 g/dl observed in our study, and reported a mean HGB value of 9.5 g/dl in the newborn. Nevertheless, this latter value was calculated as a mean of the samplings performed daily during the first week after birth; since HGB concentrations showed a decreasing trend from birth to the 7th day, the mean HGB value appears lower compared to the present results.

The PCV reference interval found in this study at birth (24.6%–45.6%) is very similar to the values reported in the adult camels, which vary between 25 and 30% but with extremes ranging from 22 to 43% (Faye and Bengoumi, 2018). Narrower ranges were reported by Martín-Barrasa et al. (2023) and by Elkhair and Elmgboul (2015), while the mean PCV values were almost superimposable to those found in the current study from d1 to d7. Since PCV provides information on the volume of circulating liquids (hemodilution, hemoconcentration), it can drastically change because of water deprivation or according to suckling time and frequency, and this might explain the wide PCV value range registered in the present study and reported in literature.

Regarding the erythrocyte indices, the MCV and MCH RIs arising from the present study were higher than those reported by Martín-Barrasa et al. (2023) and by Elkhair and Elmgboul (2015). Higher MCV values in newborns are reported also in the human species; erythrocytes are markedly macrocytic at birth, then the MCV begins to fall after the first week (Fonseca et al., 2021). Reference intervals for MCHC appear instead lower than previously reported in adult and newborn dromedary camels (Martín-Barrasa et al., 2023; Elkhair and Elmgboul, 2015). All

Table 1
Hematological variables in newborn dromedary calves at birth (d0), at 24 h (d1), and at 3 (d3) and 7 (d7) days after birth: median values, minimum-maximum values (min-max), and reference intervals (RIs) with 90% confidence interval (CI) for lower and upper limits.

Variable	Unit	d0					d1				
		Median	Min-Max	RIs	90% CI lower limit	90% CI upper limit	Median	Min-Max	RIs	90% CI lower limit	90% CI upper limit
RBC	$\times 10^6/\mu L$	8.9	7.2–14.5	**6.3-13**‡	5.9–6.8	11.7–14.2	8	5.7–13.3	**6.1–11.6**‡	5.8–6.5	10.2–12.8
HGB	g/dL	13.8	10.2–21.0	**8.7–18.6**	7.3–10	17.1–20.1	12.1	9–19.9	**9–19.7**	9–10.4	15.1–19.9
PCV	%	31.3	23–43	**24.6–45.6**‡	23.5–25.9	40.1–52.5	28.3	20.9–45	**21–44.5**§	20.9–24	34–45
PLT	$\times 10^3/\mu L$	516	266–1091	**146–941**	66–230	852–1026	382	207–627	**170–634**	123–218	582–683
MCV	fL	34.5	32–38	**30.8–38.8**#	30.5–31.7	38.1–39.4	35	31–38	**31–36**§	31–32	36–36
MCH	pg	15	13.7–17.3	**13.7–17.3**§	13.7–14.1	16.1–17.3	15	13.6–16.1	**13.7–16.3**	13.5–14	16.0–16.5
MCHC	g/dL	42.8	37.3–46.9	**39.1–46.8**	38.3–40.0	45.9–47.6	43.7	39.8–47	**39.9–46.6**	39.2–40.6	45.9–47.3
WBC	$\times 10^3/\mu L$	23.5	7.4–41	**9.4–40.6**	6.2–12.8	36.4–43.4	19.8	11.1–32	**10.6–29.8**	8.7–12.6	27.7–31.9
LYMPH	$\times 10^3/\mu L$	3.4	0.8–5.9	**1.1–5.8**‡	0.7–1.6	5.1–6.4	2.4	1.6–4.9	**1.6–4.9**#	1.4–1.7	4.6–6.3
NEUT	$\times 10^3/\mu L$	19.4	6.2–36.4	**6.6–35.7**	2.5–8.7	31.4–38.0	16.2	8.8–27.8	**8.8–27.8**#	8.1–10.3	23.7–28.9
MONO	$\times 10^3/\mu L$	0.5	0.1–1.1	**0.1–1.2**#	0–0.1	1–1.3	0.3	0.1–1.2	**0.1–1.2**§	0.1–0.2	0.9–1.2
EOS	$\times 10^3/\mu L$	0.4	0.1–1.91	**0.1–1.6**#	0–0.1	1.2–2	0.5	0.1–2.1	**0.1–2.1**#	0.1–0.1	1.4–2.3

Reference intervals are calculated by standard method on untransformed data
#reference interval derived from a standard method with a Box-Cox transformation of the data.
‡reference interval derived from a robust method with a Box-Cox transformation of the data.
§ non-parametric reference interval.

these erythrocyte indices showed no significant changes according to age or sex.

The higher PCV interval and its mean value registered at birth are in line with the higher RBC count and HGB concentrations observed in the same sampling time. Regarding the age-related variations, a significant decrease in mean RBC, HGB, and PCV was observed during the first week of life; this finding was also reported in the newborn of other species such as i) horse and pony foals (Madeiros et al., 1971; Jeffcott, 1977; Sato et al., 1979; Harvey et al., 1984; Waelchli et al., 1984) ii) donkey foals (Veronesi et al., 2014), and iii) bovine calves (Probo et al., 2012). Increased erythrocyte destruction, decreased erythrocyte production, and hemodilution have been hypothesized to explain such a decrease (Harvey et al., 1984). When looking at the literature on camel species, age was found to significantly affect blood variables by some authors (Mutugi et al., 1993; Omer et al., 2006) while other authors did not observe any age influence on them (Durand and Kchouk, 1959; Chartier et al., 1986).

The platelet count reference interval observed in the present study was wider than the one previously reported in adults by Martín-Barrasa et al. (2023), particularly for the maximum values, and this is in agreement with what was found also in newborn cattle compared to adult (Strous et al., 2021); regarding sampling time, PLT mean values showed a trend similar to those of RBC, HGB, and PCV, with a decrease from birth to d1 and d3, but then increased significantly at d7.

Regarding WBC, a range between 9.7 and 20.1 $10^3/mm^3$ has been described by different authors (reviewed in Faye and Bengoumi, 2018) with a rise from birth up to 2 years (Petrelli et al., 1982). In general, it has been reported that camels have a higher WBC count than domestic ruminants (Higgins, 1984). The WBC mean values of the present study were similar to those previously reported (Faye and Bengoumi, 2018) but higher than values reported by Martín-Barrasa et al. (2023). Moreover, a wider WBC range was found at birth compared to the ranges already reported (Elkhair and Elmgboul, 2015), and this could be related also to the timing of colostrum intake since colostrum is an important source of leukocytes. In the present study, a decrease in total WBC was registered on d3 compared to the previous days, with a subsequent rise on d7; this decrease at d3 sampling was present in all the leukocyte subpopulations, while the subsequent increase at d7 was mainly due to increases in the lymphocyte and monocyte populations. According to some authors, in comparison to other domestic herbivores, camelids have a characteristic predominance of neutrophils which might represent up to 60% of WBC as compared with the lymphocytes' predominance observed in cattle and small ruminants (Schalm et al., 1975). The dominance of neutrophils among camel blood leukocytes turns out, on average, in a very high neutrophils to lymphocyte ratio of 5:1 compared to a NLR of 1:2 usually found in domestic ruminants (Roland et al., 2014). Discrepancies in this finding are reported, and they could be due to a physiological neutrophilia induced by the catecholamines release during the blood sampling procedure (Tornquist, 2022). In the present study, the neutrophils were predominant at birth, but their % and absolute number decreased during the first week, while the opposite occurred for the lymphocytes, which made NLR significantly decrease. Differences with adult dromedaries RIs (Martín-Barrasa et al., 2023) are therefore present both for lymphocytes and neutrophils sub-populations. It was suggested that the initially high dromedary calf NLR reflects the pro-inflammatory nature of newborn camel immune responses, and a shift toward mature and correctly polarized immune responses takes place in the very first period after birth (El Sheikh et al., 2020).

Sex is one of the factors that has an influence on many hematological variables in humans as well as in animals, and it should be therefore considered in order to ensure an accurate interpretation of the blood parameters in domestic animals (Yaqub et al., 2013). Many studies reported no sex effect on RBC, HGB, and PCV in the dromedary (Petrelli et al., 1982; Abdalla et al., 1988; Mutugi et al., 1993). Contrarily, differences between females and males were observed in the present study, as RBC, HGB, PCV, PLT, WBC count, NEUT count, and NLR were found to be higher in the female group compared to the male one, and only at d7. Similar differences were reported also by Abdalmula et al. (2019) who found significantly higher values of HGB, PCV, erythrocyte osmotic fragility, MCV, MCH, and neutrophil and monocyte populations, in adult female camels compared to adult males. Martín-Barrasa et al. (2023) also found lower HGB, PCV, and RBC mean values in male camels compared to those in females and the same was registered in free-ranging Roe Deer (*Capreolus capreolus*) (Karpiński et al., 2023); in literature, it was reported that different HGB concentrations and PCV values in females may be associated with the activity of female sex hormones (Weiss and Wardrop, 2011). The differences detected in the present study between males and females at d7 suggest possible differences in the maturational and growth process between males and females also in the dromedary calves, although it is not possible to speculate about the origin of these differences. The presence of a sex influence on total WBC count or differential leukocyte composition is still debated; Martín-Barrasa et al. (2023) found higher WBC count in males than in females, in contrast to what has been observed in the present study; however only animals older than 1 year were included for

d3					d7				
Median	Min-Max	RIs	90% CI lower limit	90% CI upper limit	Median	Min-Max	RIs	90% CI lower limit	90% CI upper limit
8.1	6–11.2	**6.0–11.5**	5.7–6.4	10.7–12.4	8.2	6–13	**4.9–11.7**	4.0–5.8	10.7–12.8
12.1	9–16.4	**8.8–16.2**[‡]	8.1–9.6	15.4–17.0	12.7	9.2–19.9	**9.5–19.6**[‡]	9.2–10.0	17.5–22.1
27.9	21–38	**20.3–36.7**[§]	18.7–22.1	34.9–38.5	29	21.7–47	**21.7–46.9**	21.7–23.2	38.9–47
387	197–735	**190–705**[#]	163–222	622–796	614	222–1152	**222–1147**[§]	222–245	1032–1152
35	32–37	**32–37**[§]	32–32	36.9–37	35	31–36	**31–36**[§]	31–32	36–36
15.3	13.6–16.2	**13.8–16.4**	13.5–14.1	16.1–16.7	15.2	13.4–16	**13.4–16**[§]	13.4–14.4	15.9–16
44	40.1–47.6	**40.1–47.5**[§]	40.1–40.7	45.9–47.6	43.8	41–47.2	**40.8–46.7**	40.2–41.5	46.1–47.4
15.0	3.7–25.1	**2.9–25.9**	0.6–5.3	23.4–28.4	18.8	4.1–36.9	**4–35.8**[#]	1.7–6.6	31.2–40.6
2.4	0.7–4	**0.7–4**	0.6–1.2	3.5–4.2	3.4	1–5.6	**1–5.6**	0.4–1.4	5.2–6.3
11.9	2.8–20.6	**2.8–20.6**	0.4–4.1	18.6–22.6	14.2	3.1–31	**3.1–27.7**[#]	2.3–3.4	24.5–31.7
0.4	0–1.1	**0–1.1**[§]	0–0.1	0.9–1.1	0.8	0–2.2	**0–2.2**[§]	0–0.2	1.9–2.2
0.4	0–2.6	**0–2.6**[§]	0–0.1	1.4–2.6	0.2	0–1.4	**0.1–1.4**[#]	0.1–0.1	0.9–1.9

sex comparison in their study, and this could have played a role in the diversity of results with the present ones. The higher concentration of neutrophils in females found in the present study, and the consequently higher NLR, is in agreement with other previous findings (Nassar et al., 1977; Mohammed et al., 2008; Abdalmula et al., 2019).

Variations caused by sampling collection, related to the stress of capture and immobilization, may have affected the results, but all these procedures have to be considered an inherent challenge in studies involving free-ranging animals. Regarding the sampling procedure, the relatively small body size of the newborns and their quiet temperament allowed the veterinarians to perform blood puncture in a standing position, thus reducing restraint time and stress to the minimum. Hematological values are known to be influenced by geographical location, season, climate, day length, nutritional status, physiological status of individual animal and other non-genetic factors (e.g. parasitism); all efforts were made to standardize sampling conditions and homogenize the population.

For RIs generation, the guidelines for veterinary species (Friedrichs et al., 2012) were followed; therefore, strict inclusion criteria were applied for excluding sick animals or animals potentially affected by subclinical diseases. The application of these inclusion criteria brought to the exclusion of 6 newborns and a few blood samples affected by alterations that may have caused pre-analytic errors.

Finally, a bootstrap method was used to detect the CI in the present study. A critical drawback of this approach is that the 90% CIs can be very wide if the sample size is small (Ceriotti et al., 2009), while it is recommended that the width of the 90% CI of reference limits should not exceed the width of the RI by 20% (Harris and Boyd, 1995). Nevertheless, this represents the best statistical strategy for determining RIs in a small size population, the latter representing the main limitation of the present study. As reported by Geffré et al. (2011), the acceptability of RIs when examining a small number of individuals is based on comparing these reference values to a larger, more comprehensive original study. However, this was not feasible in the current study, since no other hematological RIs in dromedary newborn calves during the first week of life were available in the literature.

5. Conclusions

The manifold different results arising from studies on dromedaries

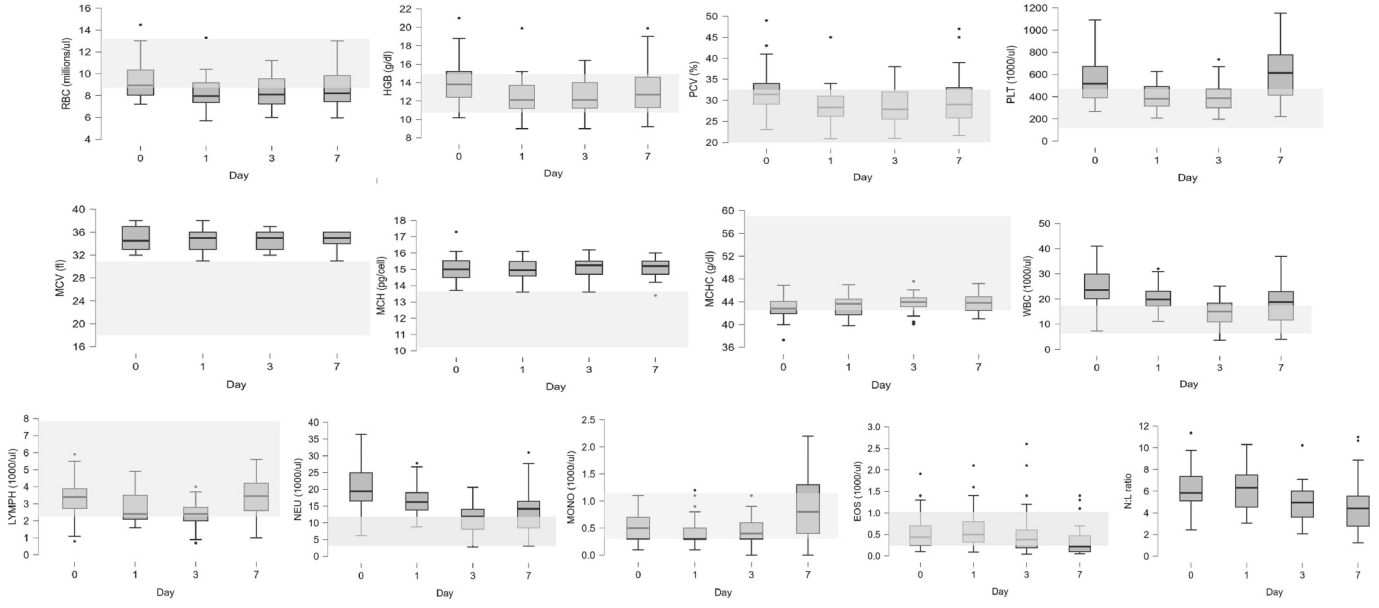

Fig. 1. Hematological Reference Intervals in the 47 dromedary newborn calves at birth (day 0), at 24 h (day 1), and at 3 (day 3) and 7 (day 7) days after birth. Boxes indicate the I–III interquartile interval, the horizontal line corresponds to the median, and vertical lines are the limits of outlier distribution. Near outliers are indicated by the symbols "•". The gray areas display the reference intervals reported in Canary Islands' adult dromedary camels by Martin-Barrasa et al. (2023).

Table 2
Hematological variables (mean ± SD) in the female, male, and total group of newborn dromedary calves at birth (d0), at 24 h (d1), and at 3 (d3) and 7 (d7) days after birth.

Variable	Unit	d0				d1				d3				d7			
		Female ($n = 18$)	Male ($n = 29$)	Total ($n = 47$)	*P*	Female (n = 18)	Male (n = 29)	Total (n = 47)	*P*	Female (n = 18)	Male (n = 29)	Total (n = 47)	*P*	Female (n = 18)	Male (n = 29)	Total (n = 47)	*P*
RBC	$\times 10^6/\mu L$	9.73±2.03	9.17±1.55	9.40±1.76^{a}	ns	8.79±1.08	8.07±1.45	8.29±1.37 ^{b}	ns	8.96±1.38	7.91±1.19	8.31±1.35	ns	9.70±1.72	7.80±1.02	8.59±1.64	**
HGB	g/dL	14.8±2.8	13.6±2.0	14.1±2.4 ^{a}	ns	13.3±1.4	12.0±2.1	12.4±2.0 ^{b}	ns	13.5±1.8	11.9±1.6	12.5±1.8	ns	14.6±2.6	11.8±1.5	12.9±2.7	***
PCV	%	34.3±6.8	31.9±4.6	32.9±5.6 ^{A}	ns	30.0±3.1	28.4±4.5	28.7±4.2 ^{B}	ns	30.5±4.0	27.4±3.6	28.6±4.0	ns	33.5±6.0	26.9±3.3	29.62±5.6	***
PLT	$\times 10^3/\mu L$	577±214	519±179	543±266 ^{a}	ns	447±109	381±111	402±113 ^{bB}	ns	447±128	370±123	398±129 ^{bB}	*	712±235	532±216	607±239^{C}	*
MCV	fL	34.7±1.5	35.0±2.2	34.8±1.9	ns	34.3±1.3	35.0±2.2	34.7±1.9	ns	34.1±1.5	34.7±1.6	34.5±1.6	ns	34.3±1.5	34.5±1.5	34.5±1.5	ns
MCH	pg	15.3±0.8	14.9±0.7	15.1±0.7	ns	15.11±0.6	14.9±0.6	15±0.6	ns	15.1±0.6	15.1±0.7	15.1±0.6	ns	15.0±0.5	15.2±0.6	15.1±0.6	ns
MCHC	g/dL	43.5±1.9	42.6±1.8	43±1.9	ns	44.1±1.7	43.0±1.5	43.3±1.6	ns	44.2±1.6	43.6±1.4	43.8±1.5	ns	43.6±1.5	43.8±1.4	43.8±1.4	ns
WBC	$\times 10^3/\mu L$	23.91±9.28	24.49±7.00	24.26±7.86^{aA}	ns	21.96±4.07	19.19±4.75	20.15±11.1 ^{A}	ns	15.61±5.33	13.78±5.70	14.47±3.7 ^{‡B}	ns	22.13±6.95	15.43±7.27	18.08±4.1$^{‡\ b}$	*
LYMPH	$\times 10^3/\mu L$	3.11±1.29	3.60±1.02	3.4±1.14 ^{‡A}	ns	2.47±0.58	3.08±1.04	2.86±0.95	ns	2.32±0.70	2.41±0.75	2.38±0.72^{B}	ns	3.5±1.19	3.28±1.19	3.37±1.18 ^{‡A}	ns
NEUT	$\times 10^3/\mu L$	20.05±8.11	20.21±6.57	20.15±7.11 ^{A}	ns	18.45±3.60	15.41±4.09	16.47±4.15^{AC}	ns	12.60±3.57	10.77±4.92	11.44±4.52 ^{BCE}	ns	17.67±6.17	10.94±5.86	13.6±6.78 ^{B}	*
MONO	$\times 10^3/\mu L$	0.44±0.28	0.54±0.27	0.5±0.28 ^{A}	ns	0.31±0.11	0.47±0.29	0.41±0.25 ^{A}	ns	0.49±0.31	0.43±0.23	0.45±0.26 ^{A}	ns	0.96±0.60	0.81±0.56	0.86±0.57 ^{B}	ns
EOS	$\times 10^3/\mu L$	0.49±0.32	0.53±0.43	0.51±0.39	ns	0.85±0.57	0.48±0.25	0.61±0.42^{a}	ns	0.90±0.70	0.30±0.20	0.52±0.53	*	0.51±0.42	0.21±0.17	0.34±0.33^{b}	ns
NLR	–	6.2±2.0	5.9±2.0	6.2±2.0 $^{‡\ ac}$	ns	7.7±1.6	5.4±0.3	6.2±2.0^{aA}	**	5.7±1.6	4.5±1.5	4.9±1.6 ^{‡bc}	ns	6.2±2.7	3.5±1.6	4.5±2.4^{bB}	**

***P*:** indicates significant differences between females and males within the same sampling time; *:$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns: not significant. ‡: indicate differences in the total group among sampling times with $p < 0.05$>; a,b,c: indicate differences in the total group among sampling times with $p < 0.01$. A,B,C: indicate significant differences in the total group among sampling times with $p < 0.001$.

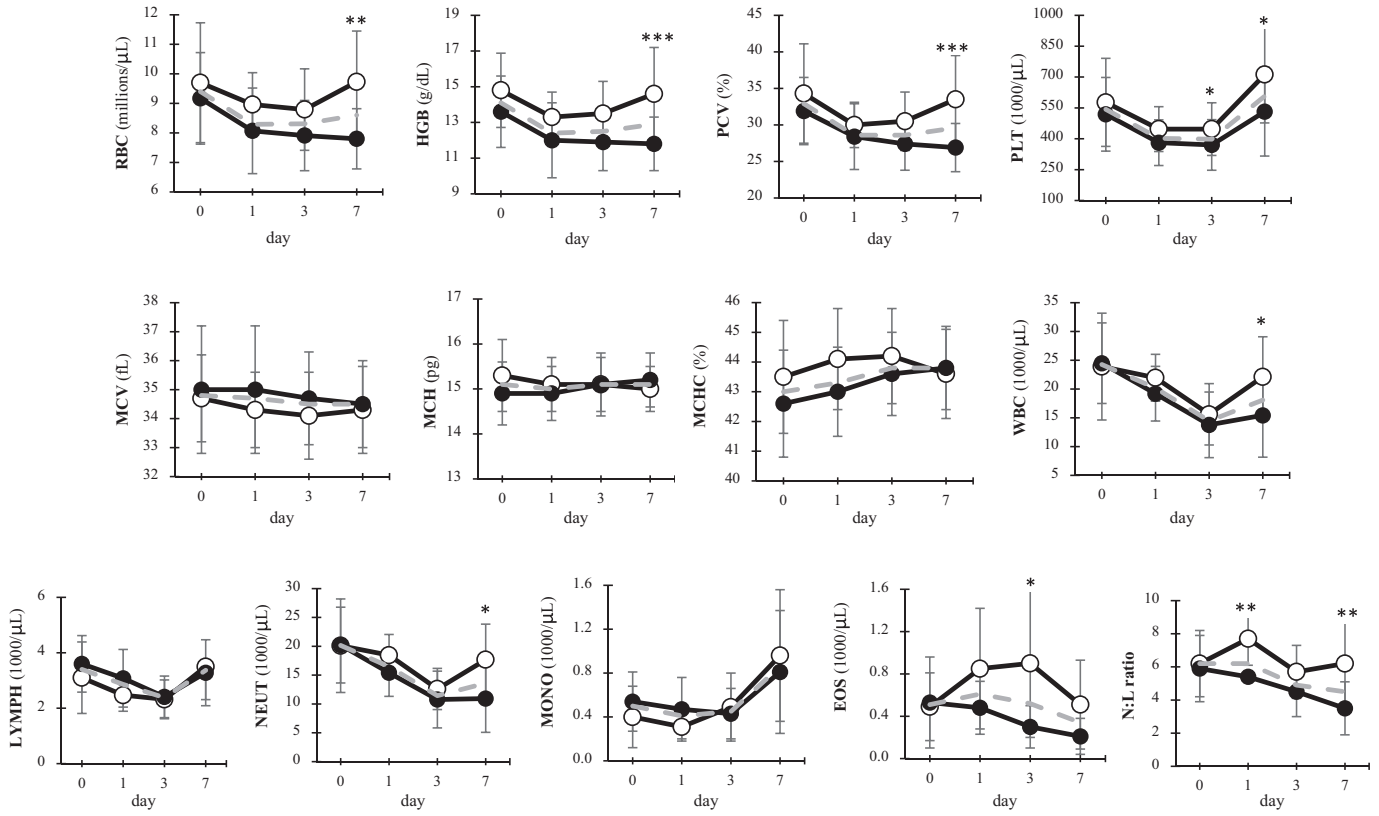

Fig. 2. Mean (±SD) hematological variables in female (○) and male (●) newborn dromedary calves, and mean values in the total group (**- - -**) at birth (day 0), at 24 h (day 1), and at 3 (day 3) and 7 (day 7) days after birth. *, **, ***; indicate significant differences between females and males within the same sampling time; *:$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

underline how heavily the hematological variables are influenced by a variety of factors including age, sex, geographical environment, management, and genetic and analytic methods in this species. This study therefore represents the first fundamental step toward the desirable generation of RIs from a larger sample size of newborn dromedary calves; the present results will aid in finding an evidence-based approach to the diagnosis, treatment, and monitoring of diseases in newborn dromedary calves, ensuring a better quality of veterinary assistance in the dromedary camel species.

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Ethics approval

The study was performed in compliance with welfare guidelines and with the regulations for the use of animals for research purposes. The experimental protocol was approved by the ethic committee of the Department of Veterinary Medicine, University of Bari Aldo Moro (approval number 15/2022).

Authors' contributions

Conceptualisation: DM, MP. Methodology: JM, TKO, AAR. Writing/preparation of original draft: DM, MP, JF. Writing, review and editing: DM, MP, JM, JF. Supervision, project administration and funding acquisition: TKO, AAR, DM, MP. All authors have read and agreed to the published version of the manuscript.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors did not used generative AI and AI-assisted technologies.

CRediT authorship contribution statement

Davide Monaco: Conceptualization, Project administration, Supervision, Writing – original draft, Writing – review & editing. **Jole Mariella:** Methodology, Writing – review & editing. **Jasmine Fusi:** Writing – original draft, Writing – review & editing. **Taher Kamal Osman:** Methodology, Project administration, Supervision. **Ahmed Abdel Rauf:** Methodology, Project administration, Supervision. **Monica Probo:** Conceptualization, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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