

Caracterización de perfiles hematológicos en razas caninas mediante análisis multivariados

Characterization of hematological profiles in dog breeds using multivariate analysis

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Palabras claves:

Hematología, análisis de componentes principales, raza, edad, sexo.

Keywords:

Hematology, principal component analysis, breed, age, sex.

Resumen

Introducción. El estudio hematológico es la base del diagnóstico clínico veterinario, este contribuye con información objetiva para la determinación de enfermedades en canes y un tratamiento médico preciso. **Objetivo.** Es la caracterizar los perfiles hematológicos en razas caninas mediante análisis multivariados. **Metodología.** Consistió en la recolección de muestras de sangre de siete perros de distintas razas y edades, analizando diversos parámetros hematológicos mediante técnicas de laboratorio. Los resultados se analizaron utilizando estadísticos de comparación de medias y el Análisis de Componentes Principales (ACP). **Resultados.** el análisis hematológico presentó valores fuera el rango de permisibilidad, lo que conlleva a una causa patológica de anemia. Del análisis de comparación de medias se observó diferencias significativas entre sexo del animal en los parámetros de hematocritos, hemoglobina y recuento de glóbulos rojos. En el análisis multivariado se identificó cuatro componentes principales que explican el 92,56% de la varianza total en los parámetros hematológicos, revelando patrones de variación relacionados con el tamaño y concentración de glóbulos rojos, eosinófilos, linfocitos y plaquetas. Se observaron diferencias significativas en parámetros como hematocrito, hemoglobina y recuento de glóbulos rojos entre las razas estudiadas. **Conclusión.** Se concluye que existen variaciones importantes en los parámetros hematológicos entre razas caninas, lo que subraya la necesidad de considerar la raza al interpretar los resultados de análisis de sangre en perros para un diagnóstico más preciso y un tratamiento más efectivo. **Área de la ciencia:** (Medicina veterinaria)

Abstract

Introduction. Hematological analysis serves as a cornerstone in veterinary clinical diagnosis, providing objective information for disease identification and accurate medical treatment in canines. **objective.** This study aims to characterize hematological profiles in canine breeds using multivariate analysis. **Methodology.** The study involved collecting blood samples from seven dogs of different breeds and ages. Various hematological parameters were analyzed using laboratory techniques. The results were analyzed using mean comparison statistics and Principal

Component Analysis (PCA). Results. Hematological analysis revealed values outside the permissible range, suggesting an underlying pathological cause of anemia. Mean comparison analysis demonstrated significant differences between animal sexes in hematocrit, hemoglobin, and red blood cell count parameters. Multivariate analysis identified four principal components explaining 92.56% of the total variance in hematological parameters, revealing variation patterns related to the size and concentration of red blood cells, eosinophils, lymphocytes, and platelets. Significant differences were observed in parameters such as hematocrit, hemoglobin, and red blood cell count among the studied breeds. Conclusion. The study concludes that significant variations exist in hematological parameters among canine breeds, emphasizing the need to consider breed when interpreting blood test results in dogs for more accurate diagnosis and effective treatment.

Introduction

Canines exhibit a diversity of breeds and, based on this, the diversity of structural physical characteristics of the individual is established, such as: size, shape and coat; this variability also extends to the biochemical or physiological level, which includes variations in hematological parameters. Understanding these differences between breeds is crucial for the accurate assessment of canine health, the diagnosis of diseases and the development of personalized treatment strategies.

In the determination of hematological parameters, a combination of manual methods and automatic techniques is used, such as state-of-the-art equipment (Abacus cell counter) for the calculation of hematocrit and total leukocyte count, and the microscopy technique for the relative differentiation of leukocytes (Vezzani et al., 2017). These laboratory methods and techniques contribute to the efficient diagnosis of canine pathologies. Currently, with technological development, the specialty of Veterinary Medicine has improved and developed a variety of complementary clinical laboratory analyses that allow an early and effective diagnosis of many diseases that affect the canine population (Cortés et al., 2015). The liver function test is the predominant laboratory test for pathological evaluation in canines, which allows for an effective diagnosis in a simple and economical way (Pedrozo et al., 2010).

Systemic and multisystemic diseases affect the respiratory, digestive, cutaneous and nervous systems of canines and are common worldwide, with no specific treatment. These diseases cause the host's immune system to be activated, inducing an inflammatory response (Willesen et al., 2009; Daldaban et al., 2021). Another category of diseases that threaten their health are joint-related diseases, such as Osteoarthritis (OA). Especially OA is a condition that causes pain, inflammation and stiffness in many joints and commonly occurs as a consequence of joint dysplasia. Although the genetic background of certain pedigree breeds, excessive exercise, nutritional imbalances, chronic inflammation and aging are also related to the development of OA (Lee et al., 2018). Hematological analysis allows the diagnosis, treatment and prevention of these diseases.

Regarding Pedrozo et al. (2010), they indicated in their study that clinical laboratories complement the clinical examination of veterinary patients. Normal and abnormal laboratory results provide objective information for differential diagnosis, prognosis, and treatment evaluation. According to Gutiérrez (2023), evaluating the hematological status of a patient is one of the first procedures used to obtain baseline or diagnostic information that can be used to determine the health status of a pet or the cause of the disease.

The aim of this study is to characterize hematological profiles in canine breeds by means of multivariate analysis. The hematological profile was analyzed in parameter values such as: red blood cells, white blood cells and platelets, to explain the variations between small, medium and large breeds. In addition, the influence of sex and age on these parameters was investigated by means of multivariate analysis. The results are intended to establish a complete reference framework that serves as a guide for veterinarians, breeders and dog lovers in general. By understanding the hematological differences between breeds, more precise and effective medical care will be provided to our furry friends.

Methodology

A. *Animal*

The study was applied to seven dogs of different breeds and sex owned by clients admitted to the Veterinary Clinic Vetsanz for a surgical procedure. The dogs that were the object of study can be seen in Table 1, the ages of the individuals were from 1 to 12 years, as a preoperative step a complete blood count and a biochemical profile were performed. The indications for surgery were diverse, including relatively minor procedures such as skin tumor removal, as well as major abdominal and orthopedic procedures. Provided that blood samples were collected according to protocol, without exclusion criteria.

Table 1

Canine demographic characteristics

Species	No.	Race	Sex	Age (years)
CANINE	1	POODLE	Female	7
	2	Mestizo	Female	8
	3	Pug	Female	1,2
	4	English Bulldog	Female	9
	5	FRENCH P.	Male	1,8
	6	SHIH TZU	Male	1
	7	SCHNAUZER	Male	12

B. Blood sampling and handling

The "gold standard" for blood sample collection in the veterinary patient is collection of blood by an experienced technician via fresh venipuncture of the jugular vein while applying minimal stasis to minimize any activation of hemostasis associated with sampling (Palsgaard-Van et al., 2007).

The blood sampling methodology used the methodology of Paudel et al. (2023) and Brloznic et al. (2023), a blood sample was collected from the jugular vein using a 20 to 21 G cannula (Vacutainer Systems, B&D Medical Systems). Minimal compression of the vein was applied and blood was aspirated into plain vials, citrate vials containing 3.2% sodium citrate and vials containing EDTA. Immediately after collection, the tubes were inverted five times to properly mix the blood. Blood samples stabilized with EDTA and coagulation activator were transported to the Diagno Vet Veterinary Clinical Laboratory, maintaining the cold chain. The EDTA vial was used to determine: 1) red series, Hematocrit, Hemoglobin, Hemoglobin, Count by G. Rojos, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corp. Hb Concentration (MCHC), Red Cell Distribution Width (RDWc), Reticulocytic Production Index. 2) White blood cell series: Total leukocytes, Relative leukocyte formula (Band Neutrophils (%), Segmented Neutrophils (%), Eosinophils (%), Basophils (%), Lymphocytes (%), Monocytes (%)), Absolute leukocyte formula (Band Neutrophils (#), Segmented Neutrophils (#), Eosinophils (#), Basophils (#), Lymphocytes (#), Monocytes (#)). 3) Platelet Series: Platelet count. 4) Plasma protein: Total solids. EDTA-stabilized blood was used for a complete blood count, which was performed on fresh whole blood within 24 h of sampling.

C. Statistical analysis

The hematological results obtained by the laboratory were applied to the mean comparison analysis and multivariate analysis as part of this, the Principal Component Analysis (PCA) to summarize the values of hematological attributes of canine individuals that were the object of this study. Errors due to scales and parameter units were reduced, the data were standardized with zero mean and unit variance. Thus, the set of 20 hematological parameters was characterized by four new parameters (PC1, PC2, PC3 and PC4). The suitability of this analysis is verified by the total information of the original parameters retained in the principal components. These data showed higher or lower eigenvalues compared to the attribute unit, only eigenvalues \geq that explain 5% of the variation of the data were considered. When more than one parameter was selected in a single PC, correlation analysis was applied to identify their correlations (González et al., 2024).

Results

Hematological analysis clinical laboratory results

Table 2 reports the hematological results obtained from the laboratory for the seven canines. The hematocrit (HCT) concentration levels in dogs 1, 2, 3, 4, 7 are below normal limits, the same ones that presented a clinical picture of anemia, except for dogs 5 and 6 that are within normal thresholds. Similarly, the concentrations or levels of hemoglobin (Hb) and the red blood cell count of canine 1, 2, 3 and 4 are also below the recommended interval, the same ones that corroborate the picture of anemia in the animals, due to this, a decrease in oxygenation in the blood is manifested. Dog number 5 presented a concentration of Hb puy above normal parameters, indicative of anemia and dehydration. The total solids (TS) concentration in dog number 3 is below normal limits, which demonstrates poor nutrition, possible chronic infectious liver disease, malabsorption syndrome or protein loss.

The mean corpuscular volume (MCV) levels in dogs 2, 4, 5, 6, 7 are within their parameter, considered as normal, except for dogs 1 and 3 whose MCV levels are above the normal limits. Mean corpuscular hemoglobin or the hemoglobin contained in an erythrocyte in dogs confirms the lack of oxygenation generated by anemia. To the same extent, the quantitative variability of the size of circulating erythrocytes can be observed, which is classically used for the differential diagnosis of anemia.

Table 2

Comparison of hematological values obtained from venous blood in seven canines

Not of individuals	1	2	3	4	5	6	7	Range
Hematology								
blood count + observation of vector-borne pathogens								
HCT: (L/L)	*0.1	*0.16	*0.1	*0.26	0.57	0.39	0.31	0.37 - 0.55
ST: (g/L)	62	72	*34	86	78	72	83	60,00 - 79,00
Hb: (g/L)	*33	*50	*30	*86	224	133	104	120,00 - 180,00
RBC: (x10 ¹² /L)	*1.07	*2.55	*1.07	*3.99	8.85	6.37	*4.45	5.50 - 8.50
VCM: (fL)	**92.5	62.75	**93.46	65.16	65.3	62.1	70.8	60,00-77,00
HCM: (pg)	**30.8	19.61	**28.04	21.55	**25.3	20.8	23.4	19,50 - 24,50
CHCM: (g/L)	333.3	312.5	300	330.77	**388	336	331	320,00 - 360,00
RDWC: (%)	**15.7	**19.1	**15.09	**18.3	**15.2	**15.7	*9.3	12,00 - 15,00
WBC: (x10 ⁹ /L)	**67.71	*2.51	**33.38	8.35	17.61	*5.7	8.21	6,00 - 18,00
Relative leukocyte formula								
Band neutrophils (NB): (%)	**31	0	2	**4	2.12	0.9	1.8	0.00 - 3.00
Segmented neutrophils (NS): (%)	*59	70	**81	**83	68.6	**80.9	**89.7	66,00 - 77,00
Eosinophils (EOS): (%)	*1	*1	0	*1	8.2	8.09	*0.5	2,00 - 10,00
Basophils (Bas): (%)	0	0	0	0	0.8	0	0.1	0.00 - 1.00
Lymphocytes (LLN): (%)	*6	20	17	*11	17.7	*2.4	*1.9	12,00 - 30,00
Monocytes (MO): (%)	3	8	*0	*1	*2.9	7.7	6	3,00 - 10,00
Absolute leukocyte formula								
Neutrophils in band: (x10 ⁹ /L)	**20.99	0	**0.67	**0.33	**0.37	0.05	0.15	0.00 - 0.30
Segmented neutrophils: (x10 ⁹ /L)	**39.95	*1.76	**27.04	6.93	**12.09	4.68	7.37	3,00 - 11,50
Eosinophils: (x10 ⁹ /L)	0.68	0.03	0	0.08	1.11	0.47	0.04	0.10 - 1.25
Basophils: (x10 ⁹ /L)	0	0	0	0	0.08	0	0	0.00 - 0.10
Lymphocytes: (x10 ⁹ /L)	4.06	*0.5	**5.67	*0.92	3.12	*0.1	*0.15	1.00 - 4.80
Monocytes: (x10 ⁹ /L)	**2.03	0.2	0	0.08	0.51	0.4	0.5	0.15 - 1.35
PLT: (x10 ⁹ /L)	*126	*43	*120	156	*149	*79	*16	150,00 - 500,00

* Values that are below and ** Values above the maximum permissible range in hematology, Hematocrit (HCT), Total solids (TS), Hemoglobin (Hb), Red blood cell count (RBC), Mean corpuscular volume (VCM), Mean corpuscular hemoglobin (MCH), Mean corpuscular Hb concentration (MCHC), Red blood cell distribution width (RDWC), Corrected white blood cell count (WBC), Platelet count (PLT).

The results reported in Table 2, for the red blood cell distribution width (RDWC) in dogs 1 to 6, are above the established normal ranges, in which the disease called anisocytosis occurs, normally as a result of anemia. To the same extent, the quantitative variability of the size of circulating red blood cells can be observed, which is classically used for the differential diagnosis of anemia.

In the relative leukocyte formula, the band neutrophils were reported to have a value ten times higher than their maximum reference value, in dog 1, which demonstrated the inability of the bone marrow to mature red blood cells. And, dog 4, presented a value above its normal parameter. In this formula, specifically in the segmented neutrophils (NS), it was observed that dog number 1, presented a value of 59% NS, which is slightly below its normal parameter (66.00 - 77.00%), which is compatible with the immunosuppression that is manifested in the animal. And, In dogs 1, 4, 6, 7, it is manifested due to very prolonged anemia or in extreme cases compatible with a case of leukemia, particularly in dog number 7. In dog number 4, we observed a decreased value of monocytes, twice less than their normal parameter, which is compatible with the immunosuppression and neutrophilia generated.

Absolute leukocyte formula, specifically, in band neutrophils, dog number 1 presented results of $20.99 \times 10^9/L$, a value much higher than its normal limit ($0.00 - 0.30 \times 10^9/L$), the same demonstrates a strong infectious process generated by the underlying cause, anemia and immunosuppression. From dog numbers 2 to 7, values of the parameters were obtained within their normal range. The segmented neutrophils in dogs 1 and 3 we see tripling in the first and doubling in the second, of their normal parameters, product of immunosuppression, and as a side effect the collateral action of opportunistic bacteria. The values reported in table 2, of eosinophils and basophils are within their normal ranges. Platelet count may show thrombocytopenia in dogs 1, 2, 3, 5, 6, 7, compatible with non-regenerative anemia generated by bone marrow dysfunction.

Table 3 presents the results of the statistical analysis of the comparison of means, which shows the existence of significant differences between females and males in terms of hematological parameters such as: HCT, Hb and RBC at a significance level of 5%, while in the other hematological parameters there are no significant differences in terms of the sex of the animal. Statistically, due to the size of the canine population studied, it could not be inferred an early conclusion that the breed or age of the animal are involved in the risk factors for contracting diseases. The hematological studies for each experimental unit are arranged by the individual biology of the animal and will depend on many conditions such as feeding, vaccination schedule and general livelihood.

Table 3

Mean and standard deviation of hematological parameters in dog breeds differentiated by sex

Parameters	Female		Male		Total		P(<0.05)
	Average	Std. Deviation	Average	Std. Deviation	Average	Std. Deviation	
HCT: (L/L)	*0.16	0.08	*0.42	0.13	*0.27	0.17	0.019 ^a
ST: (g/L)	63.50	21.99	77.67	5.51	69.57	17.59	0.335
Hb: (g/L)	*49.75	25.72	153.67	62.61	*94.29	68.72	0.028 ^a
RBC: (x10 ¹² /L)	*2.17	1.40	6.56	2.21	*4.05	2.85	0.023 ^a
VCM: (fL)	**78.47	16.79	66.07	4.40	73.15	13.83	0.277
HCM: (pg)	**25,00	5.29	23,17	2.26	24,21	4.08	0.604
CHCM: (g/L)	*319.14	15.77	351.67	31.56	333.08	27.54	0.128
RDWC: (%)	**17.05	1.95	13.40	3.56	*15.48	3.15	0.139
WBC: (x10 ⁹ /L)	**27.99	29.67	10.51	6.28	**20.50	23,25	0.372
Band neutrophils (NB): (%)	**9.25	14.59	1.61	0.63	**5.97	11,10	0.417
Segmented neutrophils (NS): (%)	73.25	11.09	**79.73	10.60	76.03	10.53	0.471
Eosinophils (EOS): (%)	*0.75	0.50	5.60	4.41	2.83	3.65	0.074
Basophils (Bas): (%)	0.00	0.00	0.30	0.44	0.13	0.30	0.214
Lymphocytes (LLN): (%)	13.50	6.24	*7,33	8.98	*10.86	7.57	0.329
Monocytes (MO): (%)	3.00	3.56	5.53	2.43	4.09	3.18	0.342
Neutrophils in band: (x10 ⁹ /L)	**5.50	10.33	0.19	0.16	**3.22	7.84	0.425
Segmented neutrophils: (x10 ⁹ /L)	**18.92	17.76	8.05	3.75	**14.26	14.01	0.355
Eosinophils: (x10 ⁹ /L)	0.20	0.32	0.54	0.54	0.34	0.43	0.337
Basophils: (x10 ⁹ /L)	0.00	0.00	0.03	0.05	0.01	0.03	0.286
Lymphocytes: (x10 ⁹ /L)	2.79	2.49	1.12	1.73	2.07	2.21	0.371
Monocytes: (x10 ⁹ /L)	0.58	0.97	0.47	0.06	0.53	0.69	0.859
PLT: (x10 ⁹ /L)	*111.25	48.15	*81.33	66.53	*98.43	53.76	0.517

* Values below and ** Values above the maximum permissible range in hematology. ^a Significant differences for P < 0.05.

Multivariate or principal component analysis (PCA) of hematological parameters

Table 4

Pearson correlation matrix of canine hematological parameters

	HCT (L/L)	ST (g/L)	Hb (g/L)	RBC (10 ¹² /L)	VCM (fL)	HCM (pg)	CHCM (g/L)	RDWc (%)	WBC (10 ⁹ /L)		
HCT	1,000	0.568	0.995	0.996	-0.654	-0.323	0.870	-0.217	-0.471		
ST		1,000	0.527	0.584	-0.788	-0.583	0.526	-0.053	-0.523		
Hb			1,000	0.986	-0.602	-0.251	0.903	-0.200	-0.406		
RBC				1,000	-0.704	-0.388	0.847	-0.156	-0.515		
VCM					1,000	0.902	-0.391	-0.189	0.868		
HCM						1,000	0.040	-0.263	0.936		
CHCM							1,000	-0.134	-0.050		
RDWc								1,000	-0.047		
WBC									1,000		
	*NB	*NS	*EOS	*LIN	*MO	NB	NS	EOS	LIN	MO	PLT
			(%)					(10 ⁹ /L)			
*NB	-0.424	0.143	0.842	-0.070	0.194	-0.442	-0.498	0.618	-0.336	-0.207	0.121
*NS	-0.167	0.166	0.277	-0.301	0.407	-0.208	-0.632	0.161	-0.803	-0.022	-0.157
*EOS	-0.379	0.067	0.842	-0.010	0.142	-0.396	-0.433	0.675	-0.255	-0.167	0.176
*LIN	-0.451	0.143	0.863	-0.061	0.242	-0.468	-0.547	0.598	-0.385	-0.236	0.107
*MO	0.627	-0.285	-0.508	-0.007	-0.587	0.635	0.933	-0.051	0.845	0.470	0.246
NB	0.732	-0.465	-0.222	-0.040	-0.613	0.730	0.966	0.354	0.861	0.652	0.421
NS	0.025	-0.277	0.746	-0.018	0.006	0.001	-0.119	0.873	-0.076	0.213	0.359
EOS	0.027	-0.448	0.043	0.570	-0.064	0.028	-0.091	0.002	0.030	-0.135	0.443
LIN	0.903	-0.636	-0.260	-0.096	-0.474	0.907	0.985	0.335	0.752	0.807	0.404
MO	1,000	-0.680	-0.237	-0.292	-0.236	0.995	0.822	0.346	0.415	0.943	0.299
PLT		1,000	-0.133	-0.325	0.028	-0.711	-0.536	-0.616	-0.416	-0.693	-0.360
*NB			1,000	-0.075	0.290	-0.227	-0.303	0.738	-0.188	-0.036	0.209
*NS				1,000	-0.279	-0.273	-0.053	0.013	0.388	-0.395	0.307
*EOS					1,000	-0.177	-0.567	-0.050	-0.745	0.010	-0.779
*LIN						1,000	0.824	0.347	0.420	0.953	0.244
*MO							1,000	0.260	0.834	0.701	0.420
NB								1,000	0.241	0.501	0.475
NS									1,000	0.262	0.565
EOS										1,000	0.118
LIN											1,000

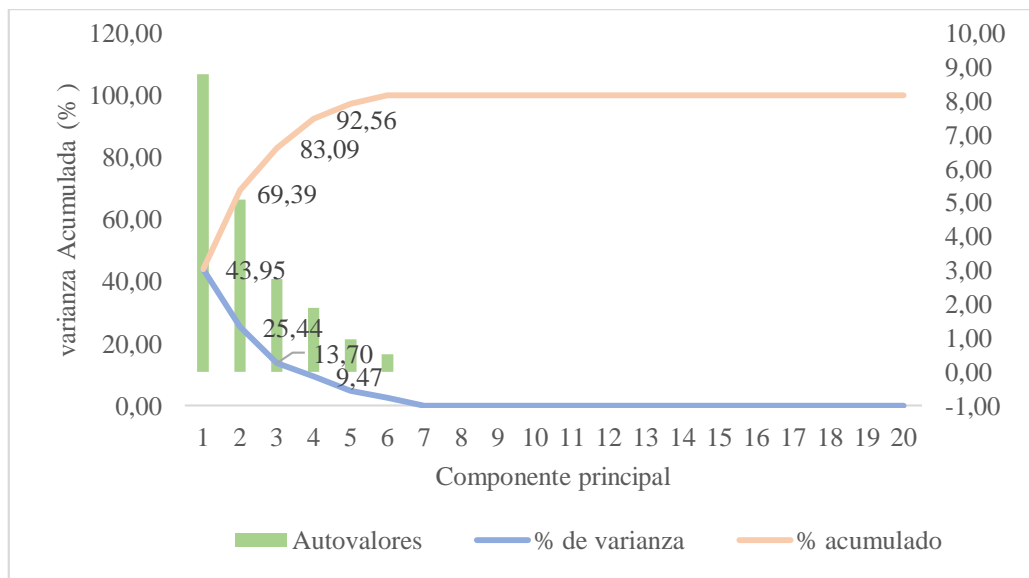
* Similar units

In the statistical analysis of the 20 hematological variables reported in Table 4, significant correlations were determined ($P \leq 0.05$). Thus, the HCT presented a positive correlation with Hb ($r=0.995$), RBC ($r=0.996$), CHCM ($r=0.870$), EOS % ($r=0.842$), Hb presented

similar behavior with the same parameters as HCT, such as: RBC ($r=0.986$), CHCM ($r=0.903$), EOS % ($r=0.842$), it is inferred that Hb and HCT are mutually dependent; RBC showed a positive correlation with CHCM ($r=0.847$), EOS % ($r=0.863$), MCV with HCM ($r=0.902$), WBC ($r=0.868$), Segmented Neutrophils: ($\times 10^9/L$) ($r=0.933$), Lymphocytes: ($\times 10^9/L$) ($r=0.845$); HCM showed a positive correlation with the same parameters as MCV are also mutually dependent parameters; CHCM shows a positive correlation with Eosinophils (EOS): ($\times 10^9/L$) ($r=0.873$); WBC with NB (%) ($r=0.903$), Band Neutrophils NB: ($\times 10^9/L$) ($r=0.907$), Segmented Neutrophils NS: ($\times 10^9/L$) ($r=0.985$), Monocytes MO: ($\times 10^9/L$) ($r=0.807$); NB % are mutually dependent with WBC; NB showed positive correlation with NS ($r=0.824$), and ST showed inverse correlation with lymphocytes: ($\times 10^9/L$) ($r=0.803$). This correlation matrix provides information about the correlation between the original parameters and the principal components. This helps to interpret the meaning of the components and how they represent the underlying structure of the data.

Figure 1

Contribution of the main components to the different canine hematological parameters analyzed



The PCA (Figure 1) was used to evaluate the interaction of hematological parameters of seven canines. The study showed that the first four principal components (PC) explained 92.56% of the total accumulated variance. PC1 (43.95%), PC2 (25.44%), PC3 (13.70%) and PC4 (9.47%) accounted for the total variance explained. For the explained and cumulative variations for each PC, 92.56% of the total variability of the data was accumulated and explained.

For the analysis and distribution of the CP, those parameters with weights >60 were identified that explained the behavior of canine hematology (Table 5). Each value in the table represents the correlation between an original parameter and a principal component. Positive signs indicate variables that increase with the component and negative signs indicate that the variable increases in the opposite direction to the component. Thus, in pCP1 the parameters are grouped (HCT, ST, Hb, RBC, VCM, HCM, WBC, NB%, band neutrophils: (x10⁹/L), segmented neutrophils: (x10⁹/L), lymphocytes: (x10⁹/L) and monocytes: (x10⁹/L)); CP2 groups the parameters (HCT, Hb, RBC, CHCM, EOS%, and eosinophils: (x10⁹/L)); for CP3 (LIN%, MO% and PLT); and for CP4 (RDWC).

Table 5

Variable loading coefficient (eigenvectors) of the four principal components for the 20 canine hematological parameters

	Component			
	1	2	3	4
Hematocrit(HCT): (L/L)	-0.712	0.665	0.073	-0.211
Total solids (TS): (g/L)	-0.663	0.255	-0.412	0.197
Hemoglobin (Hb): (g/L)	-0.654	0.716	0.117	-0.199
Red blood cell count (RBC): (x10 ¹² /L)	-0.750	0.640	0.073	-0.149
Mean corpuscular volume (VCM): (fL)	0.941	-0.083	0.078	-0.304
Mean corpuscular hemoglobin (MCH): (pg)	0.875	0.340	0.047	-0.325
Mean body Hb concentration (MCHC): (g/L)	-0.345	0.915	0.017	-0.057
Red cell distribution width (RDWC): (%)	0.043	-0.050	0.450	0.850
Corrected leukocytes (WBC): (x10 ⁹ /L)	0.945	0.306	-0.103	-0.033
Band neutrophils (NB): (%)	0.810	0.340	-0.401	0.192
Segmented neutrophils (NS): (%)	-0.523	-0.528	0.005	-0.610
Eosinophils (EOS): (%)	-0.511	0.722	0.076	0.018
Lymphocytes (LLN): (%)	0.025	-0.062	0.797	0.388
Monocytes (MO): (%)	-0.527	-0.112	-0.617	0.330
Neutrophils in band: (x10 ⁹ /L)	0.817	0.326	-0.416	0.215
Segmented neutrophils: (x10 ⁹ /L)	0.960	0.232	0.003	-0.149
Eosinophils: (x10 ⁹ /L)	0.037	0.986	0.019	0.069
Lymphocytes: (x10 ⁹ /L)	0.780	0.195	0.518	-0.229
Monocytes: (x10 ⁹ /L)	0.640	0.485	-0.574	0.153
Platelet count (PLT): (x10 ⁹ /L)	0.331	0.516	0.608	0.087

CP1 presents high negative loads in HCT, ST, Hb, RBC, CHCM, indicating that this CP is related to the size and concentration of red blood cells. And high positive loads were determined in VCM, HCM, WBC, NB%, band neutrophils: (x10⁹/L), segmented neutrophils: (x10⁹/L), lymphocytes: (x10⁹/L) and Monocytes: (x10⁹/L): indicates that the CP is influenced by the size and concentration of red blood cells.

In CP2 high positive charges were determined in HCT, Hb, RBC, EOS%, and Eosinophils: ($\times 10^9/L$) all of these parameters contribute significantly to the component, These positive loadings are with the parameters that explain the quantity and concentration of eosinophils in blood, as well as erythrocyte parameters such as HCT, Hb and RBC.

In CP3 high positive loads were determined in Lymphocytes (LIN) (%) and Platelet count (PLT) ($\times 10^9/L$) these contribute significantly to the component, they reflect the quantity and function of lymphocytes in the blood, as well as the platelet count. This may indicate that the immune system was activated, since lymphocytes are blood cells involved in the defense against infectious agents. And the negative value of Monocytes (MO) (%) indicates that it presents an inverse relationship with other parameters of CP3 such as LIN% and PLT. And in CP4 high positive loads of RDWC were determined, that is, it contributes significantly to CP4, it also indicates that this parameter may increase depending on whether other parameters associated with CP4 increase.

Discussion

Hematological analysis clinical laboratory results

The results found in this study reported in Table 2, of HCT and Hb are contrasted with those obtained by Grandía et al. (2019), who in their study indicate that 90% of the animals presented common clinical pictures of anemia, with Hb concentrations of 13.97 g/dL. And, in the mean corpuscular volume (MCV) for dogs 1 and 3 with values of 92.5 and 93.46 fL respectively, these are well above the permitted levels (60.00-77.00 fL), these animals present a disease called macrocytosis, possibly caused by deficiency of vitamins B12, folic acid, liver deficiency, and by disorders in protein formation (Aguiló, 2001).

The level of ICorrected eukocytes (WBC) of the present study differ from those determined by Viñan (2024) who indicates corresponding WBC values for young dogs of $10.4 \times 10^9/L$, adults of $11.8 \times 10^9/L$ and for geriatric dogs of $8.2 \times 10^9/L$. and according to Gutiérrez (2023), in his study indicates Comparative data between Beagles and Labrador Retrievers, that existed of Significant differences between two races in white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin, and hematocrit during the first year, and these differences were particularly prominent during the first year.

In the relative leukocyte formula, specifically in the segmented neutrophil (SN) in dogs 3, 4, 6 and 7, they are above the normal range, that is, the blood has more neutrophils than normal. The band neutrophil (NB) of dog 1 is 10 times higher than the reference values, which demonstrates the inability of the bone marrow to mature red blood cells. These results are consistent with Grandía et al. (2019), who noted the presence of hyperlobulated nuclear neutrophils and neutrophils with large bands, but cytoplasmic basophils were not

detected in abundance. This condition manifests in patients with recurrent features, in many cases they are asymptomatic, although hepatosplenomegaly is due to inflammation of the granulocyte precursor cells. Hypochromic microcytic anemia (10%), hypochromic macrocytic anemia (12%), many schistocytes and red blood cells.

Multivariate or principal component analysis (PCA) of hematological parameters

For multivariate analysis or PCA, González et al. (2024) indicate in their study that PCA is a pattern recognition technique and not a classification technique, it illustrates the relationship between the parameters in the graph and does not indicate how to classify them.

According to Bohórquez et al. (2015), there is no predisposition of breed, age or sex to present diseases. The adult canine population is more susceptible to a number of vectors than puppies, since most of these are taken to vaccination campaigns, it is believed that they have a lower risk of contracting contagious, parasitic and viral diseases. For Brloznik et al. (2014) indicate in their study that, from the evaluation of the breeds, they determined that the German Shepherd, as well as the other breeds (including crosses), did not show significant differences regarding the presence of antibodies against *E. canis*; that is, racially, the animals have the same probability of suffering from *E. canis* infection. Regardless of the hematological analysis carried out to determine the predisposition to diseases, various studies indicate that the hematological parameters are not influenced by the sex, age or breed of the animal.

Conclusions

- Hematologic findings suggest that several of the dogs in the study are experiencing anemia, potentially due to a combination of nutritional deficiencies, liver dysfunction, and underlying disease processes. Further evaluation and treatment is warranted to address these underlying issues and restore normal hematologic parameters.
- The comparison of means analysis did not reveal significant differences in hematological parameters according to sex. On the other hand, the Principal Component Analysis (PCA) showed that the first four components grouped 92.56% of the hematological parameters studied and their interrelations.

Conflict of interest

The authors declare that there are no conflicts of interest in the submitted manuscript.

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