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The effects of biological and health characteristics of dogs on intraindividual variability of blood parameters

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Abstract: Considering the great diversity among dogs and their different biological and health characteristics, as well as different treatment protocols, it has become important to assess the effects of these factors on intraindividual variability of blood parameters in dogs, which is the aim of this study. Blood samples were collected from one hundred client-owned dogs. The dogs were of different age, breed, sex, body condition, reproductive, and health status and were presented to the veterinary clinic for preventive or diagnostic procedures. The effect of age on intraindividual variation was significant for various blood parameters and it was essential to establish new reference intervals or consider using subject-based reference intervals for the senior population of dogs. Sex, breed, and body condition affected intraindividual variability of fewer parameters, so using variability indicators should be considered when interpreting those parameters rather than comparing them to the universal reference intervals. Reproductive status did not significantly contribute to the total variability of an individual and could be disregarded in the process of results interpretation. Regarding the presence of a disease or a treatment, the conclusion was that data on biological variation should be determined separately in healthy individuals, diseased animals, and animals subjected to special treatment protocols. Providing data on intraindividual variability for various types of diseases and different treatment protocols could be essential for easier monitoring of the disease and the effects of the treatment in an individual.

Key words: Variability, dogs, age, sex, breed, health

1. Introduction

In veterinary medicine, population-based reference intervals are widely used in the diagnostic process [1]. These intervals are generated based on the reference group which consists of healthy, adult, nonpregnant individuals of different age, sex, and breed [2]. Even though this approach is universally accepted, it is well known that there are dog breeds with physiologically lower or higher values of some blood parameters compared to the population-based reference intervals. This could be attributed to age, sex, and other characteristics of the animal and it is an important reason why we cannot solely rely on reference intervals when interpreting hematological and biochemical parameters of dogs [3]. In these individuals, the effect of biological variation on blood parameters should be considered. Biological variation is defined as the random fluctuation of an analyte around a homeostatic setting point and it consists of analytical variability (Va), intraindividual (Vi), and interindividual or group variability (Vg). Analytical variability of most variables is significantly lower than intra- and interindividual variability because of the high-quality control in laboratories. Intraindividual variations are changes observed in an individual during reassessment in a certain time interval or circumstances. They are defined by the intraindividual coefficient of variation (CVi). Interindividual variations are differences observed among different individuals and are defined by the interindividual coefficient of variation (CVg) [4]. Biological variation data can be used for a determination of whether the discrepancies between two consecutive measurements could be attributed to natural variability, preanalytical and analytical errors, or some other causes like disease or treatment [4].

So far, the effect of different factors has only been investigated concerning interindividual variation, and the values of blood parameters were compared between different groups of dogs. It had thereby been concluded that age, sex, breed, body condition, reproductive status, diet, living conditions, genetic factors, as well as disease and treatment affected values of routinely measured blood parameters [5]. Differences in values of blood parameters in dogs are evident from birth until old age [6,7]. Almost

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all blood parameters vary with age of the animal [8,9,10]. Sex-based variations appear in most of the hematological parameters as well as in liver and kidney profile [11,12]. Some dog breeds, such as Greyhounds, Bernese Mountain Dog, Dogue de Bordeaux, and some others, exhibit hematological and biochemical characteristics that question the validity of the commonly used reference intervals [12,13,14,15,16]. In dogs of different body condition and reproductive status, discrepancies in values of blood parameters are also present [12, 17]. In addition to internal factors, there are various effects of external factors such as disease and treatment on intraindividual variability of blood parameters, which could be used for monitoring, prognosis, and improving the health care of our patients.

As opposed to this, intraindividual biological variation studies have been limited to a small number of healthy animals of the same breed, kept at identical laboratory and dietary conditions [6,18,19]. This study will be the first to investigate the effects of biological and health characteristics on intraindividual variation of blood parameters in a larger group of dogs of different age, sex, breed, body condition, reproductive, health, and therapeutic status in different housing and dietary conditions.

2. Materials and methods

2.1. Study animals

In this research, blood samples were collected from one hundred client-owned dogs. The dogs were of different age, breed, sex, body condition, reproductive, and health status and were presented to the veterinary clinic for preventive or diagnostic procedures. The owners provided detailed history about age, health status, treatments, housing, and dietary conditions. All dogs enrolled in this study were examined by the same veterinarian. Physical examination was performed according to Virginia Tech guidelines, and ultimately the diagnosis was established. Every individual was tested for tick-borne diseases, babesiosis, ehrlichiosis, anaplasmosis, Lyme disease, as well as for adult and larval forms of *Dirofilaria immitis*.

According to age, the dogs in this research were divided into three groups. The first group consisted of 15 dogs up to 12 months of age, the second group consisted of 46 dogs from 12 to 84 months, and the third group consisted of 39 older dogs over 84 months of age. A total of 55 female and 45 male animals were included in this study. Eighteen of the females and 9 of the males were neutered. None of the remaining females were pregnant, lactating, or in heat. In terms of breed, 34 dogs were mixed breeds, while 66 were purebred. The nine-scale body condition scoring system was used according to WSAVA, which recognizes body condition scores of 4 and 5 as "ideal", from 1 to 3 as "too thin", and from 6 to 9 as "overweight" and "obese". In our examined population, 21 dogs were classified as too thin, 44 as ideal, and 35 as overweight. Based on the type of the disease, the animals were divided into 3 groups, whereby the first group consisted of 35 healthy dogs, the second group of 35 dogs that suffered from heartworm disease, and the third group consisted of 30 dogs that suffered from different diseases. Ten of the dogs had gastrointestinal problems (gastritis, enteritis, hepatitis, pancreatitis), 7 had lower urinary tract disease (cystitis and a case of a ruptured urinary bladder), 6 suffered from neoplasms (skin or mammary gland), 2 were diagnosed with immune-mediated hemolytic anemia, 2 with a vector-borne disease, and 1 dog had arthritis, bite wounds, and sebaceous adenitis. All dogs were clinically assessed as stable except for the 2 dogs that suffered from heartworm disease, 1 from hepatitis, pancreatitis, and the ruptured urinary bladder. All of the dogs but the one suffering from hepatitis recovered after treatment, but the two heartworm-infected dogs also died within 6 months of treatment due to heart failure. For treatment, the dogs were divided into 3 groups. Forty-five dogs did not undergo any treatment, 24 dogs underwent short-term treatment (3–7 days) using antibiotics, corticosteroids, nonsteroidal antiinflammatory drugs (NSAIDs), and proton pump inhibitors; and 31 dogs were subjected to long-term treatment (more than 14 days in the case of acute pancreatitis and 6 months in the case of heartworm disease) using ivermectin and the drugs mentioned previously.

All dogs were clinical cases and their blood was collected for the purposes of treatment and prophylaxis.

2.2. Hematological and biochemical analysis

Five milliliters of blood were collected from the cephalic vein of all dogs using 5-mL syringes and 22 gauge needles. Blood samples were then divided into two test tubes, one for hematological (PUTH® vacumne, K2EDTA) and the other for biochemical examination (PUTH® vacumne, Clot Activator). Serum was separated within half an hour after collecting by centrifugation at 4500 rpm for 10 min. Hettich zentrifugen EBA 200 was used. Biochemical analysis was finished within 8 h of obtaining the samples. Hematological analysis was performed 30 min after blood had been collected using the Nihon Kohden Celltacα MEK-6550 analyzer. Complete blood analysis included white blood cells (WBC), lymphocyte (LYM), neutrophil (NEU), monocyte (MON), eosinophile (EOS) count, red blood cells count (RBC), hematocrit (HCT), hemoglobin concentration (HGB), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), red blood cell distribution width (RDW), platelets (PLT), plateletcrit (PCT), mean platelet volume (MPV), and platelet distribution width (PDW). Biochemical analysis was conducted using an automatic biochemical analyzer Rayto Chemray 120Vet

using reagents manufactured by Biosystems. Biochemistry profile included total protein (TProt), albumin (Alb), globulin (Glob), blood urea nitrogen (BUN), creatinine (CREAT), glucose (Glu), total bilirubin (TBil), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), amylase (Amyl), lipase (LIPA), cholesterol (CHOL), triglycerides (TGC), calcium (Ca), and phosphorous (P).

2.3. Testing for blood parasites

Testing for *Babesia* sp. was conducted by examining blood smears stained by Diff Quick using BioGnost Bio-Diff kit and BioGnost staining procedure. Microscopic examination was performed with an Olympus CX31 microscope. Testing for thick-borne and heartworm disease was performed immediately after the blood was collected using BioNote.Inc. Anigen Rapid CaniV-4 Test Kit.

2.4. Statistical analysis

Data analysis comprised coefficient of variation calculations (CVi, CVg). Analytical variability data were obtained from our laboratory database. In addition, the animal clustering was performed according to the differences in CVi values caused by biological and health characteristics of the animals. Ultimately, the effect of biological and health factors on CVi values of the examined blood parameters was determined using a t-test (for sex, breed, and reproductive status) and ANOVA analyses (for age, BCS, disease, and treatment). SPSS statistical package was used.

Intraindividual variability was determined from two consecutive samples from the same animal and interindividual from a group of animals. Coefficient of variability was calculated using the following formula: CV (%) = (standard deviation/mean) * 100% [20].

3. Results

Biological variation data of blood parameters in dogs is shown in Table 1. Intraindividual, interindividual, and analytical variability were determined for each parameter. The results indicate that maximum precision was achieved (CVa: Cvi ≤ 0.5) for all the parameters except MCV (≤0.7) and MCH (≤0.77) and intraindividual variability was lower than interindividual variability. CVi ranged from 1.60 to 63.38.

After the significance of CVi in the pool of parameters was confirmed, the changes in CVi values were examined related to the biological and health characteristics of the animal.

Figure in the form of a heat map indicates that there are positive discrepancies of CVi for the major number of blood parameters within certain animal clusters. These clusters included a larger number of older animals with specific diseases that were treated, whereas other animal characteristics (gender, breed, body condition, and reproductive status) showed no significant discrepancies within animal clusters.

The effect of age on blood parameters was significant for CVi values of the following parameters: MCV ($p < 0.05$), MON ($p < 0.05$), PCT ($p < 0.05$), BUN ($p < 0.01$), Glu ($p < 0.01$), ALT ($p < 0.01$), ALP ($p < 0.01$), GGT ($p < 0.01$). For all the parameters except for MON, CVi exhibited the highest values in the group of dogs older than 84 months, while the highest values of CVi for MON were found in the group of dogs younger than 12 months. The effect of gender is apparent for MPV ($p < 0.05$), Alb ($p < 0.05$), and ALT ($p < 0.01$), where CVi values of these parameters are higher in males. The results are shown in Table 2.

The breed as a factor affected CVi of the following parameters: MON ($p < 0.01$), AST ($p < 0.05$), LIPA ($p < 0.05$), and P ($p < 0.05$), whereby higher values of CVi for MON appeared in mixed breed dogs and for all the other parameters, in the group of purebred dogs.

Body condition score (BCS) affected MON ($p < 0.01$) with the highest values of CVi among dogs in poorer body condition while the highest CVi values was found for LIPA ($p < 0.05$) among obese dogs.

Reproductive status did not affect Cvi values of blood parameters in this study. The results are shown in Table 3.

Disease and treatment affected CVi values of more parameters than all the other animal characteristics. In the group of dogs that suffered from hemoparasitic infections, the effect of the disease was significant for LYM ($p < 0.01$), MPV ($p < 0.05$), TBil ($p < 0.01$), ALT ($p < 0.01$), AST ($p < 0.01$), Amyl ($p < 0.01$), and CHOL ($p < 0.05$). Values of these parameters were higher in this group of animals than in other groups. In the group of dogs with various systemic diseases, significant effect of these diseases was noted for CVI values of WBC ($p < 0.05$), NEU ($p < 0.05$), PCT ($p < 0.05$), Glob ($p < 0.05$), BUN ($p < 0.01$), Glu ($p < 0.01$), ALP ($p < 0.01$), LIPA ($p < 0.01$), and GGT ($p < 0.05$) with highest values of these parameters in this group of dogs.

The effect of treatment on CVi values was found for PLT ($p < 0.05$), LYM ($p < 0.01$), PCT ($p < 0.05$), MPV ($p < 0.01$), Alb ($p < 0.05$), BUN ($p < 0.01$), CREAT ($p < 0.05$), Glu ($p < 0.05$) and Amyl ($p < 0.01$) with highest values in the group of animals receiving short-term treatment. On the other hand, in the group of dogs receiving long-term treatment, the effect of treatment was significant for CVi of WBC ($p < 0.01$), HGB ($p < 0.05$), MCV ($p < 0.01$), NEU ($p < 0.01$), TBil ($p < 0.01$), ALT ($p < 0.01$), AST ($p < 0.01$), ALP ($p < 0.01$), LIPA ($p < 0.01$), CHOL ($p < 0.05$), TGC ($p < 0.05$), P ($p < 0.01$), and GGT ($p < 0.01$), with highest values for these parameters in this group of dogs. The results are shown in Table 4.

Table 1. Interindividual (CVg), intraindividual (CVi), and analytical (CVa) variability data of blood parameters in dogs.

Parameter	Mean	CVg **	CVa**	CVi**
WBC*	13.70	66.9	3.21	18.98
RBC	6.69	20.7	1.91	7.80
HGB	155.35	22.1	2.11	7.76
HCT	45.43	21.2	1.62	7.69
MCV	68.07	5.7	1.13	1.60
MCH	23.24	7.9	2.21	2.84
MCHC	341.51	6.0	1.32	2.93
PLT	298.78	54.1	8.19	27.07
LYM	2.82	57.1	6.09	30.07
MON	0.62	90.5	5.10	40.08
EOS	0.30	131.6	4.40	63.38
NEU	9.94	68.9	5.42	22.01
RDW	12.91	12.2	1.90	4.52
PCT	0.25	49.6	2.30	22.74
MPV	8.85	33.7	0.99	10.17
PDW	15.45	6.6	0.95	3.41
TProt	66.67	17.8	1.92	7.49
Alb	30.27	19.9	2.10	6.40
Glob	36.56	82.0	3.01	12.17
BUN	7.46	119.0	1.62	23.18
CREAT	76.41	117.3	3.33	27.43
Glu	5.32	34.3	1.90	17.32
TBil	10.57	81.6	1.55	27.30
ALT	74.93	188.6	2.31	34.63
AST	62.24	159.8	2.26	25.74
ALP	128.85	181.8	4.11	30.26
Amyl	815.61	64.6	5.25	22.95
LIPA	124.54	68.3	6.11	28.30
CHOL	5.77	40.8	1.62	15.27
TGC	0.98	72.4	1.93	25.99
Ca	2.68	27.4	1.71	8.22
P	1.75	61.4	1.63	16.16
GGT	12.10	255.4	2.14	43.01

*WBC- White blood cells, RBC- Red blood cells, HGB- Hemoglobin concentration, HCT- Hematocrit, MCV- Mean cell volume, MCH- Mean corpuscular hemoglobin, MCHC- Mean cell hemoglobin concentration, PLT- Platelets, LYM- Lymphocyte count, MON- Monocyte count, EOS- Eosinophile count, NEU- Neutrophil count, RDW- Red blood cell distribution width, PCT- Plateletcrit, MPV- Mean Platelet Volume, PDW- Platelet distribution width, TProt- Total protein, Alb- Albumin, Glob- Globulin, BUN- Blood urea nitrogen, CREAT- Creatinine, Glu- Glucose, TBil- Total bilirubin, ALT- Alanine aminotransferase, AST- Aspartate aminotransferase, ALP- Alkaline phosphatase, Amyl- Amylase, LIPA- Lipase, CHOL- Cholesterol, TGC- Triglycerides, Ca- Calcium, P- Phosphorous, GGT- Gamma-Glutamyl Transferase.
**CVg – Interindividual (group) coefficient of variation, CVa- Analytical coefficient of variation, CVi – intraindividual coefficient of variation.

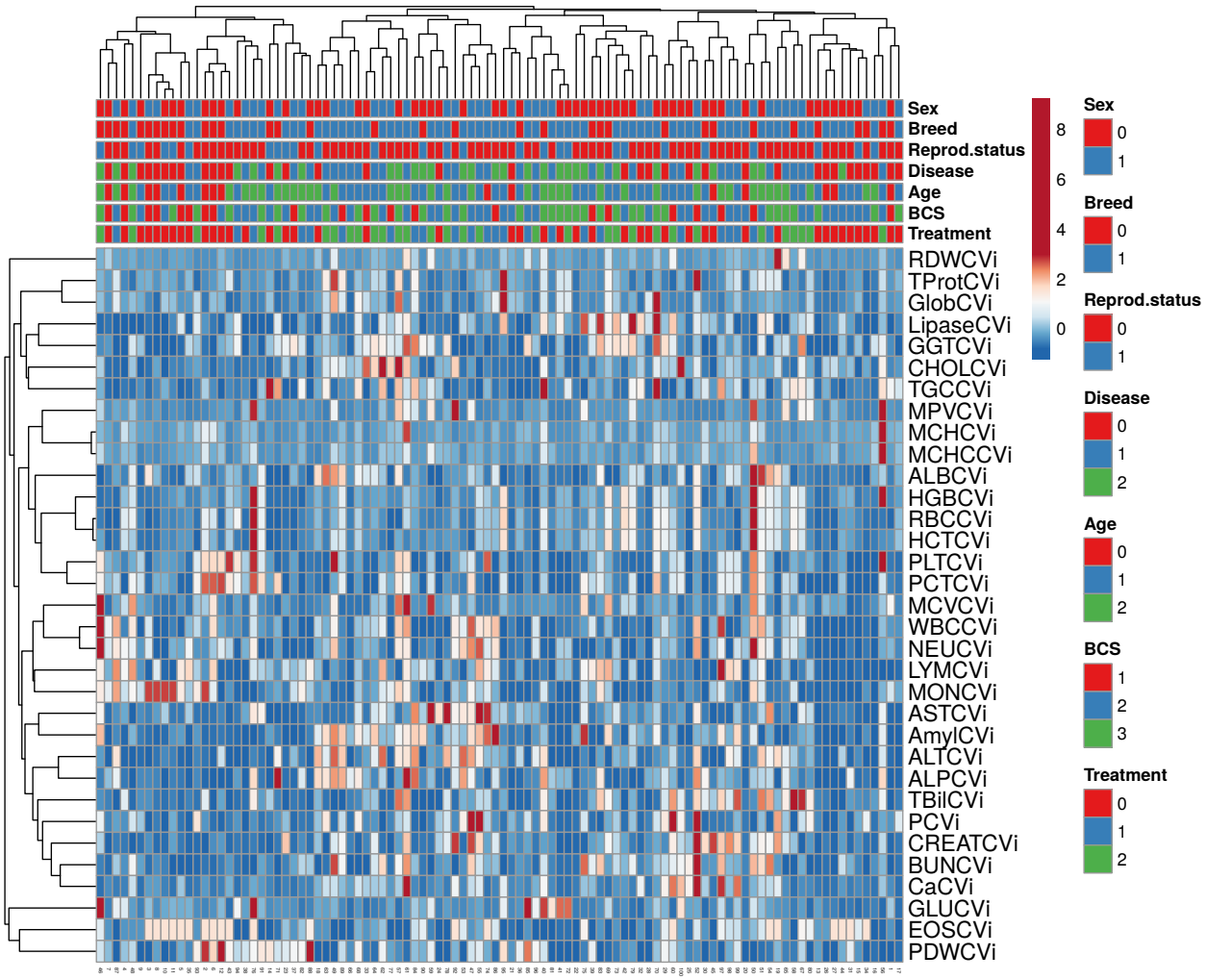

Figure. Heat map of intraindividual coefficient of variation CVi values expression in animal clusters related to animal characteristics. *Legend: Intraindividual coefficient of variation (CVi) of Red blood cell distribution width (RDWCVi), Total protein (TProtCVi), Globulin (GlobCVi), Lipase (LipaseCVi), Gamma-Glutamyl Transferase (GGTCVi), Cholesterol (CHOLCVi), Triglycerides (TGCCVi), Mean Platelet Volume (MPVCVi), Mean corpuscular hemoglobin (MCHCVi), Mean cell hemoglobin concentration (MCHCCVi), Albumin (ALBCVi), Hemoglobin (HGBCVi), Red blood cells (RBCCVi), Hematocrit (HCTCVi), Platelets (PLTCVi), Plateletcrit (PCTCVi), Mean cell volume (MCVCVi), White blood cells (WBCCVi), Neutrophils (NEUCVi), Lymphocytes (LYMCVi), Monocytes (MONCVi), Aspartate aminotransferase (ASTCVi), Amylase (AmylCVi), Alanine aminotransferase (ALTCVi), Alkaline phosphatase (ALPCVi), Total bilirubin (TBilCVi), Phosphorous (PCVi), Creatinine (CREATCVi), Blood urea nitrogen (BUNCVi), Calcium (CaCVi), Glucose (GLUCVi), Eosinophils (EOSCVi), Platelet distribution width (PDWCVi). **Animal characteristics – Sex, breed, reproductive status, disease, age, body condition score (BCS), treatment

4. Discussion

By analyzing intraindividual variability it was established that CVi values were significantly higher for PLT, LYM, and MON, and lower for EOS in this study compared to the research by Bourges-Abella et al. [6]. For all the other hematological parameters, inconsiderable differences in CVi values were found between these studies. In biochemical profile, CVi values were higher in this study than those in Ruaux et al.'s study [18] for all the parameters except for TBil and TGC because of the heterogeneous population of dogs included.

When it comes to investigating the effect of biological characteristics on CVi, it was concluded that age had a significant effect on CVi values which were high for MON, PCT, BUN, Glu, ALT, ALP, and GGT (> 25%), and low for MCV (2.29%) in the group of older dogs. This implies that old age triggers different pathophysiological compensatory mechanisms which affect organic functions and consequently lead to changes in laboratory findings [10]. Thus, reference intervals should be reevaluated in older animals and subject-based reference intervals should be used if possible. In young dogs (≤1 year) significant

Table 2. The effect of age and sex on intraindividual coefficient of variation CVi.

	AGE**					SEX***			
Parameter	Mean 0-12 months N = 15	Mean 12-84 months N = 46	Mean > 84 months N = 39	F	Sig.	Mean Female N = 55	Mean Male N = 45	F	Sig.
WBCCVi*	18.16	16.08	22.72	1.62	0.20	16.58	21.92	2.42	0.12
RBCCVi	4.86	6.99	9.88	1.80	0.17	6.96	8.81	0.91	0.34
HGBCVi	4.09	7.62	9.33	1.35	0.26	6.19	9.68	2.75	0.10
HCTCVi	4.96	6.59	10.04	2.01	0.14	7.08	8.43	0.46	0.50
MCVCVi	1.50	1.05	2.29	3.56	0.03	1.62	1.58	0.01	0.93
MCHCVi	2.69	3.19	2.49	0.12	0.89	2.20	3.63	1.11	0.29
MCHCCVi	2.27	3.10	2.96	0.10	0.91	2.14	3.89	1.90	0.17
PLTCVi	31.34	19.47	34.38	2.86	0.06	23.31	31.66	1.91	0.17
LYMCVi	27.53	26.75	34.96	1.27	0.28	30.27	29.84	0.01	0.93
MONCVi	62.34	31.99	41.06	4.10	0.02	37.57	43.16	0.57	0.45
EOSCVi	93.12	59.60	56.41	3.15	0.05	67.50	58.35	0.79	0.38
NEUCVi	22.56	17.24	27.43	2.73	0.07	18.78	25.97	3.14	0.08
RDWCVi	2.23	2.97	7.23	1.94	0.15	2.92	6.47	2.52	0.12
PCTCVi	23.27	17.48	28.74	3.41	0.04	21.98	23,67	0.17	0.68
MPVCVi	3.63	9.57	13.40	1.48	0.23	6.54	14.61	4.59	0.03
PDWCVi	3.94	3.19	3.46	0.34	0.71	3.41	3.41	0.00	1.00
TProtCVi	4.26	7.79	8.38	0.87	0.42	7.26	7.76	0.06	0.81
AlbCVi	3.75	5.44	8.54	3.19	0.05	4.83	8.31	5.92	0.02
GlobCVi	7.12	12.65	13.54	0.81	0.45	14.37	9.48	2.09	0.15
BUNCVi	9.35	21.23	30.81	4.99	0.01	20.53	26.43	1.51	0.22
CREATCVi	17.69	24.67	34.42	2.06	0.13	22.46	33.49	3.38	0.07
GluCVi	11.07	11.18	26.96	5.52	0.01	16.36	18.48	0.19	0.67
TBilCVi	17.12	26.04	32.70	1.29	0.28	24.40	30.84	0.96	0.33
ALTCVi	12.75	33.97	43.83	4.44	0.01	26.43	44.66	6.89	0.01
ASTCVi	16.51	27.63	27.06	0.92	0.40	21.85	30.49	2.30	0.13
ALPCVi	8.36	29.12	40.04	5.31	0.01	25.06	36.62	3.00	0.09
AmylCVi	12.34	28.21	20.83	3.01	0.05	21.97	24.15	0.22	0.64
LIPACVi	16.39	31.31	29.34	2.00	0.14	32.89	22.70	4.02	0.05
CHOLCVi	7.55	17.38	15.75	1.82	0.17	13.01	18.04	2.04	0.16
TGCCVi	15.33	27.18	28.68	1.33	0.27	26.53	25.33	0.05	0.83
CaCVi	3.07	8.66	9.69	1.73	0.18	6.77	10.00	1.81	0.18
PCVi	6.72	17.52	18.19	2.19	0.12	14.30	18.44	1.15	0.29
GGTCVi	13.58	47.12	49.48	6.66	0.00	43.68	42.19	0.04	0.84

* Intraindividual coefficient of variation (CVi) of White blood cells (WBCCVi), Red blood cells (RBCCVi), Hemoglobin concentration (HGBCVi), Hematocrit (HCTCVi), Mean cell volume (MCVCVi), Mean corpuscular hemoglobin (MCHCVi), Mean cell hemoglobin concentration (MCHCCVi), Platelets (PLTCVi), Lymphocytes (LYMCVi), Monocytes (MONCVi), Eosinophiles (EOSCVi), Neutrophils (NEUCVi), Red blood cell distribution width (RDWCVi), Plateletcrit (PCTCVi), Mean Platelet Volume (MPVCVi), Platelet distribution width (PDWCVi), Total protein (TProtCVi), Albumin (AlbCVi), Globulin (GlobCVi), Blood urea nitrogen (BUNCVi), Creatinine (CREATCVi), Glucose (GluCVi), Total bilirubin (TBilCVi), Alanine aminotransferase (ALTCVi), Aspartate aminotransferase (ASTCVi), Alkaline phosphatase (ALPCVi), Amylase (AmylCVi), Lipase (LIPACVi), Cholesterol (CHOLCCVi), Triglycerides (TGCCVi), Calcium (CaCVi), Phosphorous (PCVi), Gamma-Glutamyl Transferase (GGTCVi).
**Age - 0 - 12 months, 12 - 84 months and > 84 months
***Sex - Male, Female

Table 3. The effect of breed, body condition score, and reproductive status on intraindividual coefficient of variation CVi.

Parameter	BREED**				BODY CONDITION SCORE (BCS)***					REPRODUCTIVE STATUS****			
	Mean Mixed breed	Mean Purebred	F	Sig.	Mean Low BCS	Mean Ideal BCS	Mean High BCS	F	Sig.	Mean Intact	Mean Neutered	F	Sig.
	N=34	N=66			N=21	N=44	N=35			N=73	N=27		
WBCCVi*	19.28	18.83	0.02	0.90	24.80	17.86	16.90	1.57	0.21	20.17	15.77	1.30	0.26
RBCCVi	7.38	8.01	0.10	0.76	9.70	6.86	7.84	0.62	0.54	7.77	7.88	0.00	0.96
HGBCVi	7.94	7.66	0.01	0.90	9.05	7.95	6.73	0.32	0.72	8.24	6.46	0.55	0.46
HCTCVi	7.61	7.73	0.00	0.96	10.12	6.46	7.77	0.98	0.38	7.69	7.69	0.00	1.00
MCVCVi	1.63	1.58	0.01	0.91	1.73	1.60	1.52	0.06	0.94	1.65	1.46	0.14	0.71
MCHCVi	4.47	2.00	3.09	0.08	3.15	3.43	1.92	0.52	0.60	3.10	2.13	0.41	0.52
MCHCCVi	4.30	2.22	2.44	0.12	2.80	3.63	2.12	0.55	0.58	3.07	2.52	0.15	0.70
PLTCVi	28.59	26.29	0.13	0.72	27.39	31.18	21.70	0.96	0.38	30.50	17.80	3.59	0.06
LYMCVi	32.88	28.62	0.67	0.42	41.47	27.03	27.06	2.96	0.06	30.51	28.88	0.09	0.77
MONCVi	61.80	28.90	21.62	0.00	64.69	29.92	38.09	7.21	0.00	40.77	38.22	0.09	0.76
EOSCVi	73.86	57.98	2.18	0.14	82.57	62.68	52.76	2.29	0.11	65.41	57.90	0.42	0.52
NEUCVi	22.77	21.62	0.07	0.79	27.51	19.89	21.39	1.02	0.37	22.07	21.86	0.00	0.96
RDWCVi	2.80	5.40	1.22	0.27	3.37	4.01	5.84	0.40	0.67	5.19	2.69	0.99	0.32
PCTCVi	25.48	21.33	0.94	0.33	26.79	20.96	22.54	0.58	0.56	24.66	17.56	2.45	0.12
MPVCVi	11.61	9.43	0.29	0.59	6.63	13.79	7.75	1.45	0.24	11.72	5.98	1.80	0.18
PDWCVi	3.79	3.21	0.80	0.37	3.49	3.68	3.02	0.45	0.64	3.64	2.80	1.49	0.23
TProtCVi	5.83	8.34	1.29	0.26	8.55	7.52	6.81	0.18	0.84	8.07	5.92	0.83	0.36
AlbCVi	4.95	7.14	2.06	0.15	8.53	4.81	7.11	2.15	0.12	6.58	5.90	0.17	0.68
GlobCVi	9.27	13.66	1.51	0.22	10.92	10.80	14.64	0.57	0.57	12.83	10.40	0.40	0.53
BUNCVi	17.32	26.21	3.14	0.08	26.81	21.99	22.52	0.30	0.74	25.08	18.07	1.69	0.20
CREATCVi	24.90	28.73	0.36	0.55	29.52	32.87	19.33	2.07	0.13	29.43	22.02	1.19	0.28
GluCVi	16.67	17.65	0.04	0.85	14.05	17.83	18.63	0.25	0.78	15.77	21.49	1.10	0.30
TBilCVi	26.97	27.47	0.01	0.94	27.47	26.82	27.79	0.01	0.99	30.53	18.57	2.67	0.11
ALTCVi	25.11	39.54	3.80	0.05	27.13	37.12	36.01	0.60	0.55	34.99	33.68	0.03	0.87
ASTCVi	17.18	30.15	4.80	0.03	20.37	29.75	23.92	0.87	0.42	27.15	21.93	0.66	0.42
ALPCVi	27.76	31.55	0.28	0.59	30.08	29.56	31.26	0.02	0.98	30.79	28.84	0.07	0.80
AmylCVi	19.16	24.90	1.37	0.25	26.61	20.40	23.96	0.55	0.58	24.16	19.67	0.73	0.39
LIPACVi	21.11	32.01	4.17	0.04	23.28	23.08	37.88	3.97	0.02	31.16	20.59	3.42	0.07
CHOLCVi	10.70	17.63	3.57	0.06	14.89	15.93	14.68	0.05	0.95	16.94	10.77	2.45	0.12
TGCCVi	27.46	25.23	0.14	0.71	22.30	21.98	33.24	1.86	0.16	26.00	25.96	0.00	1.00
CaCVi	5.16	9.80	3.44	0.07	11.80	8.14	6.18	1.45	0.24	9.53	4.70	3.25	0.07
PCVi	10.69	18.98	4.30	0.04	14.98	14.76	18.64	0.44	0.65	16.92	14.13	0.41	0.52
GGTCVi	33.80	47.76	3.48	0.07	43.46	38.69	48.17	0.68	0.51	41.00	48.44	0.85	0.36

* Intraindividual coefficient of variation (CVi) of White blood cells (WBCCVi), Red blood cells (RBCCVi), Hemoglobin concentration (HGBCVi), Hematocrit (HCTCVi), Mean cell volume (MCVCVi), Mean corpuscular hemoglobin (MCHCVi), Mean cell hemoglobin concentration (MCHCCVi), Platelets (PLTCVi), Lymphocytes (LYMCVi), Monocytes (MONCVi), Eosinophiles (EOSCVi), Neutrophils (NEUCVi), Red blood cell distribution width (RDWCVi), Plateletcrit (PCTCVi), Mean Platelet Volume (MPVCVi), Platelet distribution width (PDWCVi), Total protein (TProtCVi), Albumin (AlbCVi), Globulin (GlobCVi), Blood urea nitrogen (BUNCVi), Creatinine (CREATCVi), Glucose (GluCVi), Total bilirubin (TBilCVi), Alanine aminotransferase (ALTCVi), Aspartate aminotransferase (ASTCVi), Alkaline phosphatase (ALPCVi), Amylase (AmylCVi), Lipase (LIPACVi), Cholesterol (CHOLCCVi), Triglycerides (TGCCVi), Calcium (CaCVi), Phosphorous (PCVi), Gamma-Glutamyl Transferase (GGTCVi).

**Breed – Mixed breed, Purebred

***BCS - Body condition score; Low BCS = (< 3), Ideal BCS = 4 - 5 and High BCS = 6 - 9

****Reproductive status – Intact, Neutered

Table 4. The effect of disease and treatment on intraindividual coefficient of variation (CVi).

	DISEASE**					TREATMENT***				
Parameter	Mean Healthy	Mean Heartworm disease	Mean Systemic disease	F	Sig.	Mean Without treatment	Mean Short-term	Mean Long-term	F	Sig.
	N=35	N=35	N=30			N=45	N=24	N=31		
WBCCVi*	11.78	22.11	23.73	5.21	0.01	11.97	21.90	26.89	8.49	0.00
RBCCVi	5.23	7.67	10.94	2.95	0.06	5.46	11.08	8.65	2.96	0.06
HGBCVi	4.55	9.17	9.84	2.59	0.08	4.61	10.26	10.38	3.85	0.02
HCTCVi	5.22	7.35	10.98	2.90	0.06	5.38	10.55	8.82	2.52	0.09
MCVCVi	0.98	1.67	2.24	2.78	0.07	0.90	1.41	2.76	7.58	0.00
MCHCVi	2.09	4.14	2.21	1.01	0.37	2.14	1.90	4.59	1.55	0.22
MCHCCVi	2.02	3.81	2.94	0.69	0.51	2.04	2.66	4.42	1.32	0.27
PLTCVi	18.70	32.08	30.99	2.13	0.12	18.29	35.05	33.63	3.67	0.03
LYMCVi	19.02	37.71	34.07	6.19	0.00	21.18	40.72	34.74	6.35	0.00
MONCVi	43.06	34.55	43.07	0.60	0.55	39.53	43.86	37.96	0.18	0.84
EOSCVi	76.99	55.97	56.15	1.94	0.15	68.58	73.59	47.94	2.17	0.12
NEUCVi	14.12	22.92	30.16	5.51	0.01	14.21	24.89	31.11	7.48	0.00
RDWCVi	5.07	4.76	3.59	0.15	0.86	4.56	2.85	5.75	0.45	0.64
PCTCVi	15.74	22.72	30.93	4.89	0.01	15.91	30.00	27.03	5.19	0.01
MPVCVi	3.23	14.46	13.27	3.80	0.03	3.44	16.89	14.75	5.67	0.00
PDWCVi	3.13	3.73	3.35	0.34	0.71	3.26	4.03	3.15	0.66	0.52
TProtCVi	4.53	7.93	10.42	2.69	0.07	4.91	10.36	9.01	2.67	0.07
AlbCVi	4.47	7.83	6.98	2.03	0.14	4.28	8.36	7.95	3.64	0.03
GlobCVi	7.51	11.52	18.37	3.52	0.03	8.03	12.73	17.74	3.16	0.05
BUNCVi	12.20	27.65	30.78	6.41	0.00	13.54	33.32	29.34	7.73	0.00
CREATCVi	18.59	36.07	27.65	3.06	0.05	19.16	37.85	31.36	3.56	0.03
GluCVi	8.93	12.94	32.20	9.81	0.00	11.96	28.59	16.37	3.94	0.02
TBilCVi	15.22	41.19	25.18	6.17	0.00	15.38	29.98	42.52	7.20	0.00
ALTCVi	17.46	45.81	41.64	7.19	0.00	18.96	38.68	54.26	11.14	0.00
ASTCVi	11.83	33.80	32.55	7.20	0.00	12.22	34.72	38.41	11.18	0.00
ALPCVi	15.33	33.74	43.63	6.75	0.00	16.19	39.89	43.25	8.37	0.00
AmylCVi	9.89	31.59	28.10	10.27	0.00	9.94	35.23	32.34	17.07	0.00
LIPACVi	17.50	29.49	39.53	6.69	0.00	20.08	29.24	39.51	5.78	0.00
CHOLCVi	10.66	21.09	13.86	3.36	0.04	10.38	15.99	21.82	4.15	0.02
TGCCVi	19.08	28.80	30.76	1.72	0.18	19.46	21.18	39.18	5.54	0.01
CaCVi	5.47	11.22	7.94	2.06	0.13	5.25	10.14	11.05	2.63	0.08
PCVi	11.41	18.86	18.57	1.66	0.19	10.65	13.39	26.31	7.20	0.00
GGTCVi	30.16	48.60	51.47	3.69	0.03	30.99	41.97	61.26	7.38	0.00

* Intraindividual coefficient of variation (CVi) of White blood cells (WBCCVi), Red blood cells (RBCCVi), Hemoglobin concentration (HGBCVi), Hematocrit (HCTCVi), Mean cell volume (MCVCVi), Mean corpuscular hemoglobin (MCHCVi), Mean cell hemoglobin concentration (MCHCCVi), Platelets (PLTCVi), Lymphocytes (LYMCVi), Monocytes (MONCVi), Eosinophiles (EOSCVi), Neutrophils (NEUCVi), Red blood cell distribution width (RDWCVi), Plateletcrit (PCTCVi), Mean Platelet Volume (MPVCVi), Platelet distribution width (PDWCVi), Total protein (TProtCVi), Albumin (AlbCVi), Globulin (GlobCVi), Blood urea nitrogen (BUNCVi), Creatinine (CREATCVi), Glucose (GluCVi), Total bilirubin (TBilCVi), Alanine aminotransferase (ALTCVi), Aspartate aminotransferase (ASTCVi), Alkaline phosphatase (ALPCVi), Amylase (AmylCVi), Lipase (LIPACVi), Cholesterol (CHOLCCVi), Triglycerides (TGCCVi), Calcium (CaCVi), Phosphorous (PCVi), Gamma-Glutamyl Transferase (GGTCVi).

**Disease- Healthy group, Heartworm disease group, Systemic disease group (most commonly gastrointestinal problems, lower urinary tract disease, neoplasms, and less frequently Immune-Mediated Haemolytic Anaemia and Babesiosis)

***Treatment- Without treatment group, Short-term treatment group- 3-7 days using antibiotics, corticosteroids, nonsteroidal antiinflammatory drugs (NSAIDs), and proton pump inhibitors, Long-term treatment group - more than 14 days in the case of acute pancreatitis using previously mentioned groups of drugs and 6 months in the case of heartworm disease using ivermectin

effect of age was found only for Mon (62.34%), which matched with the data by Bourgès-Abella et al. [6].

Sex affected CVi for MPV, Alb, and ALT whereby higher values of CVi for these parameters were determined in male dogs. Contrary to this, Connoly et al. [3] concluded that sex-based reference intervals should be used for BUN, CREAT, TBil, ALT, and PLT.

The breed as a factor of variability affected CVi for MON with the highest values in the mixed breed group and AST, LIPA, and P in the purebred group. For AST, disparity from the reference intervals which can be seen in Greyhounds, Dogue de Bordeaux, and hunting dogs could be attributed to the breed-related biological variation [13,15,16]. In Dogue de Bordeaux, this is also true for LIPA. In this research, one-third of all dogs were representatives of these breeds, which implies that for parameters in question breed-related reference intervals should be considered. The effect of breed on phosphorus variability was only seen in this research and in the study by Opoku-Agyemang et al.. MON differed in the mixed breed group of dogs but was not determined as a breed-specific parameter [21].

CVi values were affected by the body condition score for MON with the highest values in the group of dogs in lean body condition. This could be explained by the fact that animals in this group were mostly dogs suffering from chronic diseases or neoplastic processes, but there was also a possibility of analytical imprecision. In the obese dog group, the highest CVi values were found for LIPA. Obesity is often accompanied by hypertriglyceridemia and hypercholesterolemia as well as by changes in liver function which altogether cause changes in lipase activity with or without concurrent pancreatic disease [22,23].

Reproductive status did not affect CVi of blood parameters in this study, which implies that this factor did not contribute much to the total variability of an individual and could be disregarded in the process of results interpretation.

The presence of a disease and applied treatment had a significant effect on the major number of blood parameters. For instance, changes in WBC in dogs with heartworm disease originated from vascular inflammation, activation, and attraction of WBC because of the adult parasites, microfilaria, and Wolbachia [24]. Variations in platelet indices were most likely immune-mediated because platelet-bound antibodies were found in dogs suffering from this disease [25]. BUN concentration differed due to decreased renal perfusion and TProt due to increased globulin fraction [26]. Abnormalities in ALT and AST were the results of cellular damage and liver necrosis due to increased portal pressure, while changes in ALP and GGT arose from the damaged bile ducts. Hyperbilirubinemia was attributed to hemolytic anemia and hepatic damage by parasites of filaria [27]. Glu and CHOL could be altered by hypoxia [28], and Amyl as well as LIPA by decreased tissue perfusion or passive congestion of the pancreas due to subnormal cardiac output [29]. During the long-term treatment of these dogs, ivermectin was used for a period of 6 months. Due to the toxic effect of ivermectin, Panigrahi et al. [30] determined changes in HGB, HCT, MCV, MCH, EOS i LYM, as well as ALT, AST, BUN, TProt, Alb, Glob, and Glu. Dey et al. [31] detected changes in the CK, GGT, LDH (lactate dehydrogenase) and Glu, while Qureshi et al. [32] described variations in liver enzymes CHOL and TGC. The results of our study coincided in most parts with the results of these authors.

In the group of dogs with systemic diseases, there were alterations in blood parameters related to the urogenital and digestive system, as well as neoplastic diseases. In these cases, changes appeared consequently to inflammation, increased or decreased production, or elimination of the metabolites typical for these organic systems due to their pathologically altered function. The effect of short-term treatment on CVi values of blood parameters in these conditions was the result of different treatment protocols which implied the use of antibiotics, corticosteroids, nonsteroidal antiinflammatory drugs (NSAID), and proton pump inhibitors. Antibiotics rarely cause changes in blood parameters, but various studies suggest that some of them could cause hematocytopenia [33]. During short-term use of corticosteroids; leucocytosis, neutrophilia, and lymphopenia were the main hematological findings [34], and an increase in ALP and ALT was also described [35]. In dogs treated with NSAID, alteration in adhesion and aggregation function of PLT were determined [36], while in people, proton pump inhibitors caused RBC, WBC, and HGB reduction [37]. These findings partially matched with the results of our study.

5. Conclusion

By investigating the effect of biological characteristics on intraindividual variability, it was concluded that age had a significant effect on CVi values for various blood parameters and that for the older population of dogs subject-based reference intervals should be used. Sex, breed, and body condition score affected CVi of fewer parameters, so the use of variability indicators should be considered in the interpretation of those parameters rather than reference intervals. Reproductive status did not contribute much to the total variability of an individual and could be disregarded in the process of results interpretation. Moreover, providing data on intraindividual variability for various types of diseases and different treatment protocols could be essential for easier monitoring of the disease and the effects of the treatment in an individual.

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