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Age-associated and breed-associated variations in haematological and biochemical variables in young labrador retriever and miniature schnauzer dogs

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ABSTRACT

Breed, sex and age effects on haematological and biochemical variables were investigated in 24 labrador retriever and 25 miniature schnauzer dogs during the first year of life. Blood samples were taken regularly between weeks 8 and 52. White blood cell and red blood cell counts, haemoglobin concentration, haematocrit, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, platelet count as well as total protein, albumin, calcium, phosphate, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, glutamate dehydrogenase, total cholesterol, triglycerides, creatine and urea were evaluated. For all haematological and biochemical parameters, there were significant effects of age on test results. Statistically significant effects for breed and the breed×age interaction on test results were observed for most of the parameters with the exception of haemoglobin. Variations in test results illustrate growth related alterations in body tissue and metabolism leading to dynamic and marked changes in haematological and biochemical parameters, which have to be considered for the interpretation of clinical data obtained from dogs in the first year of life.

INTRODUCTION

Haematological and biochemical profiles are routinely used to monitor the health status in dogs. Established reference intervals are typically derived from data obtained in healthy adult dogs of various breeds (Lumsden and others 1979, Meinkoth and Clinkebeared 2000, Rizzi and others 2006, Schaefers 2013). However, breed-specific differences in haematological and biochemical variables are reported from recent studies in Alaskan malamutes, Siberian huskies, golden retrievers, English setters (Sharkey and others 2009), Bernese mountain dogs (Nielsen and others 2010), dachshunds (Torres and others 2014) and dogues de Bordeaux (Lavoue and others 2014). Breed-specific clinicopathological characteristics are described for certain breeds such as thrombocytopenia in cavalier King Charles spaniels (Pedersen and others 2002), elevated white blood cell (WBC) count in basenjis (Ewing and others 1972), elevated alkaline phosphatase (ALP) activity in Scottish terriers (Nestor and others 2006), elevated haemoglobin (HGB) concentration and haematocrit (HCT) in combination with lower WBC and platelet (PLT) counts in greyhounds (Shiel and others 2007, Campora and others 2011), microcytosis in shiba inus (Gookin and others 1998) and hypertriglyceridaemia in miniature schnauzers (Xenoulis and others 2007). Breed-related differences in dogs from birth to 58 days of age are reported from a large study comparing beagles, German shepherds and golden retrievers (Kuhl and others 2000, Lund and others 2000) showing lower initial red blood cell (RBC) count, lower HGB concentration, lower HCT, lower glucose levels as well as lower alanine aminotransferase (ALT) and ALP activities in the German shepherd dogs.

The effect of age on haematological and biochemical test results has been studied in basenjis, beagles, German shepherds and labrador retrievers (Kaspar and Norris 1977, Lowseth and others 1990, Strasser and others 1993, Harper and others 2003, Lawler and others 2007), suggesting that changes are most evident during the first year of life. Haematological and biochemical data in juvenile dogs however are less well documented and were often derived from studies in beagles (Earl and others 1973, Shifrine and others 1973, Wolford and others 1988, Ikeuchi and others 1991, Ishii and others

2013). Most recently changes in haematological and biochemical variables have been described in beagle, borzoi, labrador retriever and mixed breed dogs aged up to 60 days (Rosset and others 2012, Rortveit and others 2015). Both studies reported lower values for RBC, HGB, HCT and total protein as well as higher values for creatine kinase and ALP activity compared with adult reference intervals.

The aim of this study was to investigate breed, sex and age effects and their interaction in labrador retriever and miniature schnauzer aged 8–52 weeks. A previously published study evaluating the safety of vitamin A in growing dogs (Morris and others 2012) reported on some of the data used in this study. Those data were consolidated with a broader spectrum of haematological and biochemical data for this study.

To the authors' knowledge this is the first study reporting longitudinal haematological and biochemical data obtained from growing miniature schnauzers. The systematic evaluation deepens understanding of changes that should be expected in haematological and biochemical variables during the first year of life and assists the interpretation of clinical data obtained from young dogs.

MATERIALS AND METHODS

The research protocol was evaluated and approved by the WALTHAM animal welfare and ethical review committee and has been described in detail previously (Morris and others 2012).

Animals and housing

A total of forty-nine dogs, from eight litters, born at the WALTHAM Centre for Pet Nutrition entered into the study. The dogs were of two breeds, labrador retriever (24) and miniature schnauzer (25). The dogs were fully weaned by eight weeks of age. After weaning, the dogs were housed in pairs in environmentally enriched kennels. The dogs had free access to an attached outdoor area and participated daily in training and socialisation activities. All dogs were neutered between week 36 and week 52.

Clinical examination

The dogs underwent physical examination before the start of the trial and in four weekly intervals thereafter. Any blood parameters outside of the puppy reference intervals (Harper and others 2003) were referred to the veterinarian for investigation and retests were conducted within 24 hours and repeated as required for diagnosis. Only samples from dogs considered healthy at the time of sampling were entered into the database.

Diet and feeding

The base diet was a standard dry commercial recipe (Perfect Fit Junior; Mars GmbH, Verden, Germany) compliant with Fédération européenne de l'industrie des aliments pour animaux familiers (FEDIAF) recommendations for growth and reproduction (FEDIAF 2008) supplemented with various levels of vitamin A up to 104.80 µmol retinol (100,000 international unit (IU) vitamin A)/1000 kcal metabolisable energy (ME). Details of the nutrient composition and feeding regime have been provided by Morris and others (2012). Free access to drinking water was given at all times. Feeding allowances were calculated from amounts consumed during the previous week and adjusted weekly with the aim of maintaining dogs on standard growth curves with ideal body condition scores as described by Morris and others (2012).

Blood sample analysis

A 2.8 ml blood sample was taken from the jugular vein following an overnight fast of at least 16 hours at week 8, and at weeks 10, 12, 14, 16, 20, 26, 36 and 52. Blood samples were collected into a syringe and immediately distributed between tubes containing either tripotassium EDTA, lithium heparin or no anticoagulant. For haematological analysis, a 0.2 ml blood sample was deposited into a tube containing tripotassium EDTA as an anticoagulant. This was gently mixed on a roller for 10 minutes at room temperature before automated analysis (Scil Vet ABC, scil animal care, Viernheim, Germany) for WBC and RBC, HGB concentration, HCT, mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC) and PLT.

For biochemical analysis, 0.5 ml of blood was deposited into a tube containing lithium heparin as an anticoagulant. The tube was mixed gently for 10 seconds before being stored on ice for a maximum of 30 minutes. The sample was then centrifuged at 2000 *g* for 10 minutes at 4°C. The resultant plasma was pipetted into a sample cup before automated colorimetric analysis (Olympus AU400; Olympus, Tokyo, Japan). Concentrations of total protein, albumin, calcium, inorganic phosphate, total cholesterol, triglycerides, creatine and urea and activities of ALP, ALT, aspartate aminotransferase (AST) and glutamate dehydrogenase (GLDH) were measured.

Data analysis and statistics

Data were analysed by means of linear mixed model analysis including the fixed terms breed, sex, age and breed×age interaction. The model included the random term dog to take account of the correlation between measurements within an individual animal. Residuals were tested for normality using the Shapiro–Wilk test. In case of significant non-normality (at the five per cent significance level) the optimal Box–Cox transformation was determined and the Shapiro–Wilk test was again performed on the resulting residuals to assess whether the distribution of the data had been successfully normalised. For simplicity, in subsequent calculations, this optimal transform was substituted by the closest standard transformation (out of log, reciprocal, squared reciprocal or square root), this being chosen on the basis of the

value of the Box–Cox power parameter. It should be noted that residuals were tested again for normality using the Shapiro–Wilk test, before this simpler transformation was accepted as useful. In case of continued non-normality after Box–Cox transformation outliers were identified using residual plots and removed one at a time until data were normally distributed. The model was then run with both outliers included and excluded, and a comparison of the differences between the conclusions conducted. In every case the conclusions were similar–this implied that the outlying observations (all of which were biologically possible) were not influential and could be included in the model without issue. Significant breed×age interactions were investigated with a post hoc multiple comparison procedure (Tukey honestly significant difference (HSD) test).

All end points were separately subjected to Bonferroni correction to account for the presence of multiple end points; the overall significance level used was 0.05. Data are presented as means with their (Bonferroni-adjusted) 95 per cent confidence interval (CI) unless otherwise stated, and P values are reported as Bonferroni-adjusted P values. The standard error of mean (SEM) is not presented as the non-linearity of the transformation (and its inverse) implies only the means and CIs can be back-transformed to the original scale, which the authors felt was necessary to ease interpretation.

Data analyses were performed with commercially available software (Statgraphics Centurion XVI, V.16.0.07. StatPoint Technologies).

RESULTS

Clinical examination

A single labrador retriever was removed from the study at nine months of age following diagnosis of a congenital kidney defect. All data from this dog were removed from the analysis.

Haematological and biochemical testing

For all haematological and biochemical parameters, there were significant effects of age on test results. Statistically significant effects for breed and the breed×age interaction on test results were observed for most of the parameters with the exception of HGB.

Haematology

In both breeds WBC count decreased over time (P<0.01; Table 1) with most marked changes occurring between week 8 and week 14, whilst RBC count, HGB concentration, HCT, MCV and MCH increased with age (P<0.01; Table 1). PLT counts varied over time (P<0.01; Table 1) starting with an increase up to week 12 followed by a decrease until week 52.

Miniature schnauzers showed higher values for MCV and MCH compared with labrador retrievers throughout the trial (P<0.01; Table 1). MCHC fluctuated over time in labrador retriever with an initial increase between week 8 and week 16 (P<0.01; Table 1). Labrador retrievers showed higher PLT counts compared with miniature schnauzers until week 12 (P<0.01; Table 1).

Blood biochemistry

Plasma protein and albumin concentrations increased over time (P<0.01; Table 2) in both breeds with albumin reaching a plateau at week 26. Miniature schnauzers showed higher values for both plasma protein and albumin compared with labrador retrievers (P<0.01; Table 2). Urea and creatine concentrations increased until the end of the trial in both breeds (P<0.01; Table 2). Labrador retrievers showed higher creatine levels compared with miniature schnauzers from week 26 onwards (P<0.01; Table 2). GLDH activity fluctuated over time and remained higher (P<0.01; Table 2) in miniature schnauzers as of week 36. AST activity fluctuated with age (P<0.01; Table 2), initially increasing in both breeds. The ALT activity increased with age in both breeds (P<0.01; Table 2) with ALT activity reaching a plateau at week 20 in miniature schnauzers. The ALP activity decreased (P<0.01; Table 2) over time. The ALP activity in miniature schnauzers remained below the ALP activity in labrador retrievers throughout the trial (P<0.01; Table 2). Plasma calcium and phosphate concentrations decreased in both breeds until end of the trial (P<0.01; Table 2) with a marked decrease as of week 20. Plasma phosphate concentrations were higher in labrador retrievers compared with miniature schnauzers throughout the trial (P<0.01; Table 2) but no effect of breed was observed for plasma calcium concentrations (P=1.00; Table 2). Cholesterol levels varied over time (P<0.01; Table 2) with the highest values in both breeds in week 26. Plasma triglyceride levels fluctuated over time (P<0.01; Table 2) with higher plasma triglyceride levels in miniature schnauzers between week 20 and week 36 (P<0.01; Table 2).

DISCUSSION

Previously Morris and others (2012) reported a study in growing labrador retrievers and miniature schnauzers receiving various levels of vitamin A supplementation with respect to markers of vitamin A metabolism, haematological and biochemical variables and dual-energy x-ray absorptiometry. Morris and others (2012) demonstrated that different intakes of vitamin A up to 104.80 µmol retinol (100,000 IU vitamin A)/1000 kcal ME did not affect the haematological and biochemical variables. Therefore the authors did not consider vitamin A as a factor in statistical evaluation in the present study. The authors also omitted to calculate reference intervals by age and breed and to compare them with adult reference intervals in consequence of the sample size falling below recommendations for non-parametrical reference intervals as laid down in the american society for veterinary clinical pathology (ASVCP) guidelines (Friedrichs and others 2012). However, due to the scarcity of data in growing dogs the

TABLE 1: Age-specific and breed-specific results of haematological tests in miniature schnauzer and labrador retriever

Test	Unit	Breed	Age 8 weeks Mean (95% CI)	10 weeks Mean (95% CI)	12 weeks Mean (95% CI)	14 weeks Mean (95% CI)	16 weeks Mean (95% CI)	20 weeks Mean (95% CI)	26 weeks Mean (95% CI)	36 weeks Mean (95% CI)	52 weeks Mean (95% CI)	Breed P value	Age (weeks) P value	Breed×age (weeks) P value
WBC	−103/µl	LR	13.5^{a} (11.9 to 15.0)	9.49^{bc} (8.41 to 10.6)	8.85^{bc} (7.92 to 9.97)	8.76^{bc} (7.77 to 9.78)	9.03^{bc} (8.00 to 10.1)	8.76^{bc} (7.85 to 9.87)	7.39^{c} (6.62 to 8.25)	8.00^{c} (7.17 to 9.03)	8.33^{bc} (7.46 to 9.39)	1.00	<0.01	<0.01
		MS	13.2^{a} (11.8 to 14.7)	10.1^{b} (9.03 to 11.2)	10.9^{ab} (9.78 to 12.2)	8.41^{bc} (7.61 to 9.39)	8.50^{bc} (7.61 to 9.49)	7.61^{c} (6.82 to 8.41)	8.17^{bc} (7.32 to 9.12)	7.92^{c} (7.10 to 8.85)	9.12^{bc} (8.17 to 10.2)			
RBC	×106/µL	LR	4.60^{a} (4.44 to 4.78)	5.09^{b} (4.88 to 5.34)	4.97^{ab} (4.77 to 5.20)	5.31^{bc} (5.06 to 5.59)	5.48^{bc} (5.21 to 5.79)	5.79^{c} (5.48 to 6.15)	6.04^{cd} (5.69 to 6.45)	6.71^{d} (6.24 to 7.31)	6.46^{cd} (6.03 to 6.98)	<0.05	<0.01	0.36
		MS	4.68^{a} (4.52 to 4.86)	4.81^{ab} (4.63 to 5.00)	4.96^{ab} (4.76 to 5.17)	5.21^{b} (4.99 to 5.46)	5.33^{bc} (5.09 to 5.60)	5.27^{bc} (5.04 to 5.53)	5.91^{cd} (5.59 to 6.29)	6.29^{cd} (5.91 to 6.74)	6.04^{cd} (5.70 to 6.44)			
Haemoglobin	g/dL	LR	9.80^{a} (9.43 to 10.3)	10.9^{b} (10.4 to 11.5)	10.6^{ab} (10.2 to 11.1)	11.5^{bc} (11.0 to 12.2)	12.3^{cd} (11.7 to 13.0)	12.8^{cd} (12.1 to 13.5)	13.6^{d} (12.8 to 14.4)	15.5^{e} (14.5 to 16.6)	15.1^{de} (14.1 to 16.1)	0.26	<0.01	0.06
		MS	10.8^{ab} (10.3 to 11.3)	11.0^{b} (10.5 to 11.5)	11.4^{bc} (10.8 to 11.9)	12.1^{c} (11.5 to 12.7)	12.6^{cd} (11.9 to 13.2)	12.8^{cd} (12.2 to 13.6)	14.5^{de} (13.6 to 15.4)	15.1^{de} (14.3 to 16.2)	15.1^{de} (14.2 to 16.1)			
HCT	%	LR	29.9^{a} (29.0 to 31.0)	32.9^{bc} (31.6 to 34.4)	32.0^{ab} (30.7 to 33.4)	34.3^{bc} (32.8 to 36.1)	35.8^{c} (34.1 to 37.7)	38.1^{cd} (36.1 to 40.5)	40.1^{d} (37.7 to 42.9)	45.4^{de} (42.1 to 49.6)	43.9^{de} (40.9 to 47.6)	<0.01	<0.01	0.13
		MS	32.5^{b} (31.3 to 33.9)	33.2^{bc} (31.9 to 34.7)	34.4^{bc} (33.0 to 36.0)	36.5^{cd} (34.8 to 38.4)	37.6^{cd} (35.8 to 39.8)	38.0^{cd} (36.0 to 40.2)	42.9^{de} (40.2 to 46.3)	46.2^{e} (42.9 to 50.4)	45.2^{de} (42.1 to 49.1)			
MCV	fL	LR	65.1^{ab} (64.2 to 65.9)	64.6^{a} (63.7 to 65.4)	64.5^{a} (63.7 to 65.3)	65.0^{ab} (64.0 to 65.8)	65.4^{ab} (64.6 to 66.3)	65.6^{ab} (64.8 to 66.4)	66.4^{b} (65.4 to 67.2)	67.4^{b} (66.6 to 68.2)	68.1^{bc} (67.1 to 68.9)	<0.01	<0.01	<0.01
		MS	69.4^{c} (68.6 to 70.2)	69.2^{c} (68.4 to 70.1)	69.6^{c} (68.7 to 70.4)	70.1^{c} (69.2 to 70.9)	70.7^{cd} (69.9 to 71.6)	72.3^{d} (71.4 to 73.1)	72.6^{d} (71.7 to 73.4)	73.4^{de} (72.6 to 74.3)	75.0^{e} (74.1 to 75.9)			
MCH	pg	LR	21.3^{a} (20.8 to 21.7)	21.4^{a} (20.9 to 21.8)	21.4^{a} (21.0 to 21.9)	21.7^{ab} (21.3 to 22.2)	22.4^{b} (21.9 to 22.8)	21.8^{ab} (21.3 to 22.3)	22.5^{b} (22.0 to 22.9)	22.8^{bc} (22.4 to 23.3)	23.3^{bc} (22.8 to 23.8)	<0.01	<0.01	<0.05
		MS	22.9^{bc} (22.5 to 23.4)	22.8^{bc} (22.4 to 23.2)	22.8^{bc} (22.5 to 23.3)	23.1^{bc} (22.8 to 23.6)	23.5^{c} (23.0 to 24.0)	24.3^{cd} (23.8 to 24.8)	24.4^{cd} (23.9 to 24.8)	24.1^{cd} (23.6 to 24.6)	24.9^{d} (24.5 to 25.4)			
MCHC	g/dl	LR	32.7^{a} (32.1 to 33.2)	33.1^{ab} (32.5 to 33.6)	33.3^{ab} (32.7 to 33.8)	33.4^{ab} (32.9 to 34.0)	34.2^{b} (33.7 to 34.8)	33.3^{ab} (32.8 to 33.8)	33.8^{b} (33.3 to 34.4)	33.9^{b} (33.4 to 34.4)	34.3^{b} (33.7 to 34.8)	0.08	<0.01	< 0.01
		MS	33.1^{ab} (32.6 to 33.6)	33.0^{ab} (32.5 to 33.5)	32.9^{ab} (32.4 to 33.4)	33.1^{ab} (32.5 to 33.6)	33.3^{ab} (32.8 to 33.8)	33.7^{ab} (33.1 to 34.2)	33.6^{ab} (33.0 to 34.1)	32.9^{a} (32.3 to 33.4)	33.2^{ab} (32.7 to 33.8)			
Platelet	×10^{3}/µl	LR	426^{ab} (376 to 476)	429^{ab} (379 to 478)	498^{a} (448 to 547)	441^{ab} (391 to 491)	397^{b} (347 to 446)	373^{bc} (323 to 422)	311^{bc} (261 to 360)	286^{c} (237 to 336)	309^{bc} (260 to 359)	<0.01	<0.01	<0.01
		MS	288^{c} (241 to 335)	286^{c} (228 to 333)	339^{bc} (291 to 386)	352^{bc} (304 to 399)	317^{bc} (270 to 365)	353^{bc} (305 to 400)	275^{c} (227 to 322)	288^{c} (241 to 335)	259^{c} (212 to 307)			

Data are presented as means with their Bonferroni corrected 95 per cent CI

[a,b] Mean values within the same test for breeds and weeks; dissimilar superscript letters indicate significant differences within test between breeds and weeks (using Tukey's HSD method)

Data were analysed using a linear mixed model

CI, confidence interval; HCT, haematocrit; LR, labrador retriever; MCH, mean cell haemoglobin; MCHC, mean cell haemoglobin concentration; MCV, mean cell volume; MS, miniature schnauzer; RBC, red blood cell; WBC, white blood cell

TABLE 2: Age-specific and breed-specific results of biochemical tests in miniature schnauzer and labrador retriever

Test	Unit	Breed	Age 8 weeks Mean (95% CI)	10 weeks Mean (95% CI)	12 weeks Mean (95% CI)	14 weeks Mean (95% CI)	16 weeks Mean (95% CI)	20 weeks Mean (95% CI)	26 weeks Mean (95% CI)	36 weeks Mean (95% CI)	52 weeks Mean (95% CI)	Breed P value	Age (weeks) P value	Breed×age (weeks) P value
Protein	g/l	LR	46.1[a] (45.0 to 47.3)	47.3[a] (46.1 to 48.4)	47.7[ab] (46.5 to 48.8)	49.8[bc] (48.7 to 51.0)	50.2[bc] (49.1 to 51.4)	51.0[bc] (49.9 to 52.2)	52.8[c] (51.7 to 54.0)	54.0[cd] (52.8 to 55.1)	55.4[d] (54.2 to 56.5)	<0.01	<0.01	<0.01
		MS	50.7[bc] (49.6 to 51.8)	49.7[b] (48.6 to 50.8)	51.6[bc] (50.5 to 52.7)	52.1[c] (51.0 to 53.2)	53.5[cd] (52.4 to 54.6)	55.5[d] (54.4 to 56.6)	58.4[e] (57.3 to 59.5)	57.9[e] (56.8 to 59.0)	57.9[e] (56.8 to 59.0)			
Albumin	g/l	LR	22.5[a] (21.7 to 23.2)	24.1[b] (23.3 to 24.9)	25.3[bc] (24.5 to 26.1)	25.5[bc] (24.7 to 26.2)	26.2[c] (25.4 to 27.0)	26.8[cd] (26.0 to 27.6)	28.2[d] (27.4 to 29.0)	28.1[d] (27.3 to 28.9)	28.4[d] (27.6 to 29.2)	<0.01	<0.01	<0.01
		MS	26.9[cd] (26.1 to 27.6)	25.8[c] (25.0 to 26.5)	26.4[c] (25.6 to 27.1)	27.1[cd] (26.3 to 27.8)	27.9[d] (27.2 to 28.7)	28.3[d] (27.6 to 29.1)	30.7[e] (29.9 to 31.4)	30.7[e] (29.9 to 31.5)	30.6[e] (29.8 to 31.3)			
Phosphate	mmol/l	LR	2.83[a] (2.76 to 2.90)	2.78[a] (2.71 to 2.86)	2.84[a] (2.77 to 2.92)	2.82[a] (2.75 to 2.89)	2.84[a] (2.77 to 2.92)	2.72[ab] (2.64 to 2.79)	2.57[bc] (2.50 to 2.65)	2.18[d] (2.10 to 2.25)	1.81[f] (1.74 to 1.89)	<0.01	<0.01	<0.01
		MS	2.60[b] (2.53 to 2.67)	2.51[bc] (2.44 to 2.58)	2.43[c] (2.36 to 2.51)	2.45[c] (2.38 to 2.52)	2.49[bc] (2.42 to 2.56)	2.36[c] (2.29 to 2.43)	2.00[e] (1.92 to 2.07)	1.68[f] (1.61 to 1.75)	1.51[g] (1.44 to 1.58)			
Calcium	mmol/l	LR	2.80[ab] (2.76 to 2.84)	2.79[ab] (2.76 to 2.83)	2.79[ab] (2.76 to 2.83)	2.83[ab] (2.80 to 2.87)	2.80[ab] (2.76 to 2.84)	2.77[b] (2.73 to 2.80)	2.77[b] (2.73 to 2.81)	2.66[c] (2.63 to 2.70)	2.61[c] (2.57 to 2.65)	1.00	<0.01	<0.01
		MS	2.86[a] (2.82 to 2.90)	2.83[ab] (2.80 to 2.87)	2.80[ab] (2.76 to 2.84)	2.79[ab] (2.76 to 2.83)	2.85[a] (2.81 to 2.88)	2.77[b] (2.74 to 2.81)	2.77[b] (2.73 to 2.80)	2.64[c] (2.60 to 2.68)	2.62[c] (2.58 to 2.65)			
ALP	U/l	LR	424[ab] (401 to 448)	464[a] (441 to 488)	442[ab] (418 to 465)	432[ab] (409 to 455)	390[b] (367 to 414)	329[c] (306 to 353)	270[d] (246 to 293)	195[e] (171 to 218)	144[f] (120 to 167)	<0.01	<0.01	<0.01
		MS	442[ab] (420 to 464)	400[b] (378 to 423)	382[bc] (359 to 404)	375[bc] (252 to 397)	340[c] (318 to 362)	279[d] (257 to 302)	193[e] (171 to 216)	113[fg] (91.2 to 136)	72[g] (49.4 to 94.0)			
ALT	U/l	LR	19.9[a] (17.5 to 22.6)	22.0[a] (19.3 to 25.0)	24.8[ab] (21.8 to 28.2)	26.3[b] (23.1 to 30.0)	28.2[b] (24.8 to 32.1)	30.9[bc] (27.1 to 35.2)	35.5[bc] (31.2 to 40.4)	44.7[cd] (39.3 to 50.9)	48.9[d] (42.9 to 55.7)	<0.05	<0.01	<0.01
		MS	28.2[b] (25.0 to 32.1)	32.1[bc] (28.5 to 36.6)	32.8[bc] (29.1 to 37.3)	33.8[bc] (29.7 to 38.1)	37.0[c] (32.8 to 41.7)	40.9[cd] (35.9 to 46.1)	40.9[cd] (36.2 to 46.5)	47.0[cd] (41.7 to 53.5)	41.3[cd] (36.6 to 47.0)			
AST	U/l	LR	26.4[b] (24.3 to 28.8)	28.6[bc] (26.2 to 31.2)	29.4[bc] (27.1 to 32.1)	29.5[bc] (27.1 to 32.1)	30.7[bc] (28.2 to 33.4)	31.8[c] (29.1 to 34.5)	32.9[c] (29.7 to 35.5)	32.9[c] (30.3 to 35.9)	32.2[c] (29.7 to 35.2)	0.23	<0.01	<0.01
		MS	22.0[a] (20.3 to 24.0)	27.9[bc] (25.8 to 30.6)	29.1[bc] (26.6 to 31.5)	28.5[bc] (26.3 to 30.9)	30.0[bc] (27.7 to 32.5)	27.7[bc] (25.3 to 30.0)	26.6[b] (24.5 to 29.1)	24.3[ab] (22.4 to 26.3)	25.0[ab] (23.1 to 27.4)			
GLDH	U/l	LR	4.14a (3.56 to 4.85)	4.39[ab] (3.78 to 5.16)	4.76[ab] (4.10 to 5.58)	4.62[ab] (3.97 to 5.42)	4.53[ab] (3.90 to 5.26)	5.00[ab] (4.31 to 5.87)	4.44[ab] (3.82 to 5.21)	4.01[a] (3.42 to 4.66)	4.01[a] (3.46 to 4.66)	<0.01	<0.01	<0.01
		MS	4.85[ab] (4.18 to 5.64)	5.16[ab] (4.44 to 5.93)	5.42[ab] (4.66 to 6.23)	5.70[b] (4.95 to 6.62)	5.99[b] (5.05 to 7.10)	6.62[b] (5.75 to 7.69)	5.93[b] (5.10 to 6.89)	7.03[b] (6.05 to 8.08)	6.11[b] (5.26 to 7.10)			
Cholesterol	mmol/l	LR	4.93[b] (4.49 to 5.36)	5.44[bc] (5.01 to 5.87)	5.67[bc] (5.24 to 6.11)	6.38[c] (5.95 to 6.82)	6.39[c] (5.95 to 6.82)	6.44[c] (6.00 to 6.87)	6.76[c] (6.33 to 7.20)	6.13[c] (5.70 to 6.57)	5.73[bc] (5.30 to 6.17)	1.00	<0.01	<0.01
		MS	5.12[ab] (4.71 to 5.54)	4.19[a] (3.77 to 4.60)	4.81[ab] (4.40 to 5.23)	5.61[bc] (5.20 to 6.03)	5.73[bc] (5.31 to 6.14)	6.37[c] (5.95 to 6.78)	6.87[c] (6.48 to 7.29)	6.79[c] (6.38 to 7.21)	6.25[c] (5.83 to 6.66)			
Triglycerides	mg/dl	LR	55.1[ab] (46.5 to 65.4)	55.1[ab] (46.5 to 65.4)	50.9[ab] (42.9 to 60.3)	56.8[ab] (46.1 to 70.8)	66.0[bc] (55.7 to 78.3)	54.6[ab] (46.1 to 64.7)	47.5[ab] (40.0 to 56.3)	43.8[ab] (37.0 to 51.9)	42.1[a] (35.5 to 49.9)	<0.01	<0.01	<0.01
		MS	56.8[ab] (47.9 to 66.7)	53.0[ab] (44.7 to 62.2)	59.1[b] (50.4 to 69.4)	59.1[b] (50.4 to 70.1)	54.1[ab] (46.1 to 64.1)	83.1[c] (70.8 to 97.5)	75.9[bc] (64.1 to 89.19	63.4[bc] (54.1 to 75.2)	58.0[ab] (48.9 to 68.0)			

Continued

TABLE 2: Continued

Test	Unit	Breed	Age 8 weeks Mean (95% CI)	10 weeks Mean (95% CI)	12 weeks Mean (95% CI)	14 weeks Mean (95% CI)	16 weeks Mean (95% CI)	20 weeks Mean (95% CI)	26 weeks Mean (95% CI)	36 weeks Mean (95% CI)	52 weeks Mean (95% CI)	Breed P value	Age (weeks) P value	Breed×age (weeks) P value
Creatine	µmol/l	LR	36.6[a] (34.4 to 38.7)	39.5[ab] (37.4 to 41.7)	42.3[b] (40.1 to 44.4)	47.7[c] (45.5 to 49.8)	51.4[c] (49.3 to 53.6)	58.5[d] (56.4 to 60.7)	73.6[f] (71.5 to 75.8)	87.6[g] (85.5 to 89.7)	94.3[h] (92.2 to 96.5)	<0.01	<0.01	<0.01
		MS	38.9[ab] (36.8 to 40.9)	40.1[ab] (38.1 to 42.2)	44.3[bc] (42.2 to 46.3)	47.9[c] (45.9 to 50.0)	51.5[c] (49.5 to 53.6)	58.7[d] (56.7 to 60.7)	67.3[e] (65.3 to 69.3)	72.8[f] (70.7 to 74.8)	73.7[f] (71.6 to 75.7)			
Urea	mmol/l	LR	2.00[a] (1.66 to 2.35)	2.50[ab] (2.15 to 2.84)	2.82[b] (2.47 to 3.16)	3.34[bc] (3.00 to 3.69)	3.56[c] (3.21 to 3.90)	4.08[cd] (3.74 to 4.43)	4.85[de] (4.50 to 5.19)	5.39[e] (5.04 to 5.73)	6.06[e] (5.72 to 6.41)	1.00	<0.01	<0.01
		MS	2.68[ab] (2.35 to 3.01)	2.80[b] (2.47 to 3.13)	2.87[b] (2.54 to 3.20)	3.27[bc] (2.94 to 3.60)	3.77[c] (3.44 to 4.10)	4.64[d] (4.31 to 4.97)	4.84[de] (4.51 to 5.17)	5.44[e] (5.11 to 5.77)	5.45[e] (5.12 to 5.78)			

The overall significance level used was 0.05. The effect of 'breed' for AST was not significant (P=0.23).
Data are presented as means with their Bonferroni corrected 95% CI
[a,b]Mean values within the same test for breeds and weeks; dissimilar superscript letters indicate significant differences within test between breeds and weeks (using Tukey's HSD method)
Data were analysed using a linear mixed model
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; GLDH, glutamate dehydrogenase; LR, labrador retriever; MS, miniature schnauzer

data from the present study can be considered to be an important contribution for the deepened understanding of age-related and breed-related variances that should be expected in haematological and biochemical test results during the growth period.

In this study the authors found significant effects of age, breed and a breed×age interaction in haematological and biochemical tests in young labrador retrievers and miniature schnauzers between week 8 and week 52. The age-associated changes in haematological and biochemical values reported in this study are in good agreement with findings in juvenile dogs reported in previous studies (Bulgin and others 1970, Ewing and others 1972, Pickrell and others 1974, Wolford and others 1988, Ikeuchi and others 1991, Harper and others 2003, Lawler and others 2007, Ishii and others 2013), confirming the need for age-specific reference intervals for the interpretation of clinical data obtained from young dogs.

No differences were found based on sex, which correlates well with previous reports in growing dogs (Kuhl and others 2000, Lund and others 2000, Shiel and others 2007, Ishii and others 2013). Differences between male and female dogs, despite being small and probably of minor physiological significance, are reported in studies looking at adult dogs of different breeds (Pickrell and others 1974, Campora and others 2011, Lawrence and others 2013). In addition to sex, the neutering status has been reported to account for small differences leading to higher HGB concentration, MCH and MCHC in neutered dogs (Lawrence and others 2013). These differences were putatively associated with the potential direct impact of sex hormones on erythropoiesis and the indirect impact on the haematopoietic niche of the bone marrow, which might also explain the missing differences in sexually immature dogs.

The effect of neutering on haematological values in this study however remains unclear due to the fact that all dogs in this study were neutered between week 36 and week 52 and no control group of entire dogs was available.

An effect of age was found for WBC count where values rapidly decreased. Within the differential leucocyte counts a plateau was reached between week 10 and week 14. The negative correlation with age is in agreement with findings in previous studies; however some breed-related differences with regard to the time point of WBC count stabilisation are reported. A WBC count decrease until eight months of age with stabilisation at around 16 months of age was reported from a study in beagles (Bulgin and others 1970). In basenjis (Ewing and others 1972) WBC counts first increased reaching maximal values between 85 days and 120 days, with a subsequent decline to values of adult dogs. No correlation with age however was found in beagle, borzoi, labrador retriever and mixed breed dogs up to 60 days of age, which might be explained by effects of breed and sample size (Rosset and others 2012) or the automated analyser used (Rortveit and others 2015). A study in

growing greyhounds (Shiel and others 2007) aged between 5 months and 11 months reported no correlation with age, which is in good agreement with the early stabilisation found in the present study. Regardless of these breed-related differences the interpretation of WBC counts in dogs younger than six month of age requires careful interpretation as values are likely to be above or at the upper end of the adult reference intervals (Rizzi and others 2006).

RBC count, HGB concentration and HCT significantly increased in both breeds over time. This is in agreement with previous studies (Bulgin and others 1970, Ewing and others 1972, Harper and others 2003, Lawler and others 2007, Shiel and others 2007, Rortveit and others 2015) and relates to the maturation of the erythropoiesis and the positive correlation with age between RBC life span and HGB concentration (Bulgin and others 1970).

Despite a steady increase in MCV and MCH until the end of trial all values were comparable to values found in adult dogs. This might be explained by the fact that first samples were taken at eight weeks of age when the transition from fetal to postnatal erythrocytes has already taken place and MCV as well as MCH values are expected to approach adult values (Bulgin and others 1970, Shifrine and others 1973, Lund and others 2000, Rortveit and others 2015). For MCV and MCH a significant effect of breed was observed with miniature schnauzers having significantly higher MCV and MCH throughout the trial. It can be speculated whether the smaller RBC size in labrador retriever presents an adaptation to the higher metabolic rate (Brenten and others 2014) providing an increased surface area for oxygen exchange (Hawkey 1975).

PLT counts were negatively correlated with age which is consistent with findings in beagles (Ishii and others 2013). The age effect however was primarily driven by significantly higher PLT values in labrador retriever during the first three month of age, whereas the age effect was less prominent in miniature schnauzers. Despite this breed effect values for both miniature schnauzers and labrador retrievers remained within the adult reference intervals throughout the study, which is consistent with previous reports (Rosset and others 2012, Rortveit and others 2015) suggesting a low clinical relevance of the observed differences.

Plasma protein, albumin and urea concentrations were positively correlated with age, which is consistent with findings in other breeds (Ewing and others 1972, Harper and others 2003, Ishii and others 2013, Rortveit and others 2015). In miniature schnauzers total protein and albumin reached a plateau at around 26 weeks of age and remained significantly higher compared with labrador retrievers, which might be explained by the higher protein intake per kg absolute bodyweight.

Creatine significantly increased over time in both breeds, which is in good agreement with a recent study in growing labrador retriever and mixed breed dogs (Rortveit and others 2015). Labrador retriever however showed significantly higher creatine values compared with miniature schnauzers from week 26 onwards. The creatine increase over time might be a reflection of the increase in lean body mass during growth. It can be hypothesised that both the absolute higher lean body mass and the higher growth velocity (Brenten and others 2014) in labrador retrievers contributed to the significantly higher creatine values compared with values observed in miniature schnauzers after 26 weeks of age.

GLDH reference values for growing dogs have not been reported previously. The GLDH values reported in this study are consistent with a recently reported reference interval for adult dogs (Schaefers 2013), which suggests that this range can be applied to growing dogs until new data become available. Further research will be required however as differences in breed of dog, age range and analytical methodologies may impact the final reference interval.

The increase in AST activity in labrador retriever is in good agreement with previous studies in beagles reporting an increase in AST activity for up to six months of age (Wolford and others 1988, Ishii and others 2013). In the present study ALT activity increased steadily over time. An increase in ALT activity was also observed in beagles, but the ALT activity level in beagles stabilised much earlier at around three months of age (Wolford and others 1988). Whilst ALT is found predominantly in the liver, AST is found in cardiac muscle, skeletal muscle, liver and the kidneys. The age-dependent activities in both enzymes appear to correlate well with tissue growth. The higher AST activity in labrador retriever as of week 26 might be correlated with the higher absolute muscle mass.

ALP levels were negatively correlated with age which corresponds well with previous reports (Kaspar and Norris 1977, Ikeuchi and others 1991, Harper and others 2003). The high ALP activity is thought to be a result of the high bone ALP isozyme activity related to the bone turnover in growing dogs (Kramer and Hoffmann 1997). Labrador retrievers showed significantly higher ALP activities throughout the trial compared with miniature schnauzers, which is in agreement with results from a previous study looking at the differences between great danes and miniature poodles (Tryfonidou and others 2003). Plasma phosphate levels remained at a high level until four months of age in both breeds followed by a subsequent decrease until end of trial, which is consistent with results from previous studies (Pickrell and others 1974, Wolford and others 1988, Ikeuchi and others 1991). The plasma phosphate levels in labrador retriever stayed above the levels in miniature schnauzer throughout the trial. The higher plasma phosphate levels in young dogs are described as a result of the growth hormone mediated stimulation on the renal phosphate reabsorption (Mulroney and others 1989, Haramati and others 1990). Plasma calcium levels remained at a plateau until week 26 followed by a decrease until the end of trial. The decline however was less distinct in absolute values compared with the

decline in phosphate, which might be a consequence of the tight plasma calcium regulation via calcitonin and parathyroid hormone. These findings are in good agreement with a previous study in beagles (Wolford and others 1988) and a study in great danes and miniature poodles (Tryfonidou and others 2003). It can be hypothesised that the observed changes over time in plasma calcium levels are driven by the synergistic effect between passive calcium absorption (Tryfonidou and others 2002) and active absorption influenced by growth hormone stimulated production of $1,25(OH)_2D_3$ or by decreased clearance of $1,25(OH)_2D_3$ (Goff and others 1990), respectively. Two other studies in beagles however found no correlation in plasma calcium levels with age throughout the first year of life (Pickrell and others 1974, Ikeuchi and others 1991). Due to missing information about the nutritional composition of diets in these studies it can only be speculated whether this observation was associated with nutritional factors. In contrast to ALP activity and plasma phosphate concentrations it appears that plasma calcium concentrations during growth are not influenced by breed size.

Cholesterol levels increased in both breeds reaching a peak in week 26 followed by a gradual decrease until the end of trial. This compares well with findings in beagles aged between two weeks and one year as reported previously (Wolford and others 1988). In contrast a study looking at beagles aged between 6 months and 12 months (Ikeuchi and others 1991) found no significant correlation with age. Another longitudinal study in beagles (Pickrell and others 1974) reported increasing cholesterol concentrations only in females as of 11 months. In the present study however the sex×age interaction was not significant (P=1.00), which might be a consequence of the limited observation period.

Triglyceride levels in labrador retrievers showed less fluctuation during the trial period compared with miniature schnauzers. A peak triglyceride concentration was observed in miniature schnauzers in week 20 which might be a result of the peak energy intake per kg metabolic bodyweight in miniature schnauzers in week 20 as reported previously (Brenten and others 2014) leading to high intakes of dietary fat. Subsequent to this peak both energy intake and triglyceride concentration gradually decreased until end of trial. One study (Ikeuchi and others 1991) found a distinct increase in triglycerides in females and hypothesised a hormonal effect on lipase activity known from studies in rodents (Hamosh and Hamosh 1975). This effect was not found in the present study which might be a consequence of the neutering of the study dogs between week 36 and week 52.

Idiopathic hypertriglyceridaemia however is a common clinicopathological finding described in miniature schnauzers. A recent study in 192 healthy miniature schnauzers has shown a prevalence of primary hypertriglyceridaemia in 32.8 per cent of the dogs (Xenoulis and others 2007) with a significant positive correlation between triglyceride concentration and age in this breed. Ten out of the 17 miniature schnauzers remaining after completion of the study at the WALTHAM Centre for Pet Nutrition were diagnosed with hypertriglyceridaemia later on at ages between 4.5 years and 6 years. Nine miniature schnauzers had mild increases in triglyceride concentrations (109–400 mg/dl) and one had a moderate increase in triglyceride concentrations (>400–1000 mg/dl). The development of idiopathic hypertriglyceridaemia during adulthood as reported here supports the hypothesis that later development of hypertriglyceridaemia cannot be excluded in young miniature schnauzers with normal triglyceride concentrations (Xenoulis and others 2007).

CONCLUSION

The present study shows that maturation and growth in dogs is reflected in changes in haematological and blood biochemical values. The systematic evaluation of the results from this study therefore contributes to the understanding of age-related variances that should be expected in haematological and biochemical test results, which will assist practising veterinary clinicians with the interpretation of clinical data obtained from young dogs. The early growth phase clearly appears to be most critical and needs to be investigated in more depth.

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