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Holopainen, Saila

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Polycythemia in dogs with chronic hypoxic pulmonary disease

Saila Holopainen[1,2,3] | Henna P. Laurila[1] | Anu K. Lappalainen[1] | Minna M. Rajamäki[1] | Sanna J. Viitanen[1]

[1]Department of Equine and Small Animal Medicine, Faculty of Veterinary Medicine, University of Helsinki, Helsinki, Finland

[2]Department of Medical and Clinical Genetics, University of Helsinki, Helsinki, Finland

[3]The Folkhälsan Research Center, Biomedicum Helsinki, Helsinki, Finland

Correspondence
Saila Holopainen, Department of Equine and Small Animal Medicine, Faculty of Veterinary Medicine, University of Helsinki, P.O. Box 57 (Viikintie 49), 00014 Helsinki, Finland.
Email: saila.holopainen@helsinki.fi

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Abstract

Background: Prolonged tissue hypoxia caused by chronic pulmonary disease is commonly regarded as an important mechanism in the development of secondary polycythemia, but little clinical data are available to support this hypothesis.

Objective: To study the prevalence and severity of erythrocytosis accompanying chronic hypoxic pulmonary disease in dogs.

Animals: Forty-seven dogs with hypoxic chronic pulmonary disease, 27 dogs with nonhypoxic chronic pulmonary disease, and 60 healthy controls.

Methods: Dogs with chronic pulmonary disease and chronic hypoxemia (partial pressure of arterial oxygen [PaO_2] < 80 mm Hg on at least 2 arterial blood gas measurements a minimum of 1 month apart) were identified retrospectively from patient records. Association between arterial oxygen and red blood cell parameters was analyzed using Pearson's correlation coefficients and multivariable linear regression analysis.

Results: Red blood cell parameters measured at the end of the hypoxemia period were within the laboratory reference range in most dogs. In chronically hypoxemic dogs, hematocrit (Hct) was increased in 4/47 (8.5%; 95% confidence interval [CI], 0-17) dogs, erythrocyte count (Erytr) was increased in 12/47 (26%; 95%CI, 13-38) dogs and hemoglobin concentration (Hb) was increased in 3/47 (6.4%; 95%CI, 0-14) dogs. No marked polycythemia (Hct ≥65%) was noted in any of the dogs. Red blood cell parameters were not associated with the severity of hypoxemia (correlation to PaO_2: Erytr, $r = -.14$; Hb, $r = -.21$; Hct, $r = -.14$; $P > .05$ for all).

Conclusions and Clinical Importance: Polycythemia is uncommon, and usually mild if present, in dogs with chronic hypoxia caused by pulmonary disease.

KEYWORDS
dog, erythrocytosis, hypoxemia, pulmonary

Abbreviations: ANOVA, analysis of variance; B, unstandardized partial regression coefficient; BAL, bronchoalveolar lavage; CI, confidence interval; CIPF, canine idiopathic pulmonary fibrosis; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; CT, computed tomography; EPO, erythropoietin; EPOR, erythropoietin receptor; Erytr, erythrocyte count; FiO_2, fraction of inspired oxygen; Hb, hemoglobin; Hct, hematocrit; HIF, hypoxia-inducible factor 1; HIF-1α, hypoxia-inducible factor 1α; IL, interleukin; IPF, idiopathic pulmonary fibrosis; IQR, interquartile range; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; PaO_2, partial pressure of arterial oxygen; SD, standard deviation; SpO_2, peripheral capillary oxygen saturation; WHWT, West Highland white terrier.

1 | INTRODUCTION

Prolonged tissue hypoxia caused by chronic pulmonary disease is considered commonly as an important mechanism in the development of secondary polycythemia in dogs.[1-3] A deficiency in tissue oxygen leads to increased expression of hypoxia-inducible factor 1 (HIF-1) and a subsequent increase in the production of erythropoietin (EPO) by Type 1 (stellate) cells in the renal cortex.[3-6] Once EPO is released into circulation, it binds to its receptors (EPOR) on erythroid progenitor cells in bone marrow, leading to erythropoiesis and improved oxygen carrying capacity.[3,5,7] Expression of EPO and EPOR under hypoxic conditions also is controlled by HIF-1.[5,8] The net effect of hypoxemia-induced EPO production is an increase in erythrocyte numbers and blood hemoglobin concentration.[5]

Chronic hypoxemia commonly occurs in a variety of pulmonary diseases of dogs, including canine idiopathic pulmonary fibrosis (CIPF), chronic bronchitis, and other interstitial lung diseases.[9-11] As expected, polycythemia is a well-recognized sequela of chronic tissue hypoxia in dogs with right-to-left cardiac shunting.[12] Information on the prevalence and severity of erythrocytosis associated with hypoxemic lung disease in dogs however is scarce. Contradictory to expectations, erythrocytosis was not noted in 12 West Highland white terriers (WHWTs) with hypoxemic CIPF, and erythrocytosis did not develop in dogs living and training at high altitude.[13,14]

The role of secondary erythrocytosis is also complex in humans with hypoxemic lung disease because erythrocytosis is detected in only a proportion of hypoxemic patients with pulmonary disease. For example, in a previous study polycythemia was present in 62% of patients with idiopathic pulmonary fibrosis (IPF) and in 48% of the patients with pigeon breeders' lung disease.[15] In most studies of humans describing hypobaric hypoxemia at high altitudes, both red blood cell parameters and EPO production increased in relation to hypoxia.[16-18] However, in lung diseases of humans the correlation between blood EPO concentration and erythropoiesis is less clear, and normal or subnormal red blood cell parameters appear to be common despite increased blood EPO concentrations.[19]

Our aims were to investigate the prevalence and severity of erythrocytosis in dogs with chronic hypoxemia caused by pulmonary disease and to compare red blood cell parameters in hypoxemia caused by CIPF and other chronic lung diseases.

2 | MATERIALS AND METHODS

2.1 | Study population

Medical records of dogs diagnosed with chronic pulmonary disease at the University of Helsinki Small Animal Teaching Hospital between January 2011 and December 2020 were retrospectively reviewed. Dogs with chronic lower respiratory tract signs and documented chronic hypoxemia (partial pressure of arterial oxygen [PaO_2] < 80 mm Hg on at least 2 separate arterial blood gas measurements a minimum of 1 month apart) were included in the study. Included dogs had received a pulmonary diagnostic evaluation with various combinations of diagnostic imaging, bronchoscopy with bronchoalveolar lavage (BAL) and histopathology. Complete hematology and serum creatinine concentration measurement were available for the same visit.

Dogs with nonhypoxemic chronic pulmonary disease (chronic cough >2 months, $PaO_2 \geq 90$ mm Hg) that had pulmonary diagnostic evaluation, hematology, and serum creatinine concentration measurement available for the same visit were included as controls. Dogs were included in the nonhypoxemic control group if they had bronchoscopy and BAL performed and BAL cytology was indicative of inflammatory bronchial disease (neutrophilic [>7%], eosinophilic [>20%], lymphocytic [>17%], or mixed airway inflammation and no bacterial growth).

Additionally, a group of healthy control dogs lacking pulmonary disease was included. The group consisted of healthy WHWTs with no respiratory signs or abnormal respiratory findings on physical examination as well as hematology, serum biochemistry, arterial blood gases, and thoracic computed tomography (CT) performed as part of the previously published study,[20] and volunteer blood donor dogs from the University of Helsinki Canine Blood Bank with no respiratory signs, normal physical examination, unremarkable leucocyte and thrombocyte counts and serum biochemistry, and available red blood cell parameter results.

All dogs with suspected (eg, history of anorexia, vomiting, or diarrhea) or documented dehydration at the physical examination performed at the time of hematology assessment were excluded. Additionally, dogs with suspected or documented erythrocyte loss (eg, bleeding, hemolysis) or renal disease potentially affecting EPO production as well as dogs of sight hound breeds were excluded from the study.[21]

2.2 | Diagnostic examinations

A clinical diagnosis of a pulmonary disease was based on a variable combination of the following diagnostic tests: thoracic radiographs and CT (Somatom Emotion Duo, Siemens, Germany and GE LightSpeed VCT 64, GE Healthcare, Fairfield, Connecticut), bronchoscopy (GIF-N180, Olympus Europa SE&Co. KG, Hamburg, Germany), BAL fluid cytology and bacterial culture, cardiac ultrasound examination (iE33 and EPIQ7, Philips Ultrasound), fecal examination with $MgSO_4$ flotation and Baermann's sedimentation method and lung histopathology. The clinical diagnosis established by the attending clinician was verified by a review of patient records, imaging findings, laboratory results, and bronchoscopy recordings by the senior pulmonologists (MMR and SJV). Thoracic radiographs and CT images were reassessed by the senior radiologist (AKL) to ensure radiographic and CT findings were compatible with the established diagnoses.

2.3 | Sample collection and analysis

Arterial blood samples were obtained from the femoral or metatarsal artery using a 25G needle attached to a blood gas syringe and

immediately analyzed using an arterial blood gas analyzer (ABL 800 Flex analyzer, Radiometer Medical ApS, Brønshøj, Denmark). Arterial blood samples were collected at an atmospheric fraction of inspired oxygen (FiO_2) of 21% at 10 m above sea level. Hypoxemia was graded as mild when PaO_2 was 70-79 mm Hg, moderate when PaO_2 was 60-69 mm Hg, and severe when PaO_2 was <60 mm Hg. The alveolar-arterial oxygen gradient ($A\text{-}aDO_2$) was calculated at 37°C without temperature correction using the equation $A\text{-}aDO_2 = (FiO_2[Pb - PH_2O] - [PaCO_2/R]) - PaO_2$, where Pb is the barometric pressure, PH_2O is the water vapor pressure, and R (respiratory quotient) is 0.8.

Samples for hematology assays were collected into EDTA tubes with precise filling and were analyzed without delay using a high-volume hematology analyzer (Advia 2120i, Siemens AG, Erlangen, Germany). Serum biochemistry was assayed using an automated clinical chemistry analyzer (Konelab 30i Clinical Chemistry Analyzer, Thermo Scientific, Fisher Scientific Oy, Vantaa, Finland).

2.4 | Ethical and experimental animal approvals

This study consisted of a retrospective review of patient records and was not subject to ethical review. Some of the WHWTs were recruited using written and informed owner consent as part of other clinical studies at the University of Helsinki Small Animal Hospital.[13,20,22,23] These study protocols were approved by the Ethics Committee for Animal Experimentation at Helsinki University, Finland (statement numbers 5B/2008, 1/2014, 4/2018, and 13/2020) and by the Committee for Experimental Animals of Southern Finland (ESLH-2008-05403/Ym-23, HY 132-05, ESAVI/1005/04.10.03/2011, ESAVI/5794/04.10.03/2011, ESAVI/7383/04.10.07/2013, ESAVI/9116/04.10.07/2014, ESAVI/9184/04.10.07/2014, ESAVI/10906/04.10.07/2017, and ESAVI/29986/2020).

2.5 | Statistical analysis

Descriptive statistics are presented as mean ± SD for continuous and normally-distributed variables, as median and interquartile range (IQR) for skewed variables, and as frequencies and percentages for categorical variables. Normality of the variables was tested using the Kolmogorov-Smirnov and Shapiro-Wilk tests and visual inspection. Comparison between groups for non-normally distributed variables (age and weight) was conducted using Kruskal-Wallis 1-way analysis of variance (ANOVA) and comparison between groups for normally distributed variables was analyzed using 1-way ANOVA and Bonferroni correction was applied for post hoc tests. Differences between CIPF and non-CIPF dogs were evaluated using Student *t* tests. Correlations between PaO_2 and red blood cell parameters were analyzed using Pearson's correlation coefficients. The effect of hypoxemia on red blood cell parameters was studied using linear, nonlinear, and curvilinear regression analysis. Because analyzed nonlinear and curvilinear models did not fit the data better than linear regression analysis, results for linear regression analysis were reported. Separate multivariable linear regression models were created for each red blood cell parameter as dependent variable and pulmonary function and demographic covariates as independent variables. For the linear regression models, results were presented as unstandardized partial regression coefficients (B) with 95% confidence intervals (CI). Data were analyzed using commercially available software SPSS System, version 26 (SPSS Inc, Chicago, Illinois). Figures were created using GraphPad Prism, version 9 (GraphPad Software, San Diego, California). Values of $P < .05$ were considered significant.

3 | RESULTS

3.1 | Dogs

Fifty-one dogs with chronic hypoxemia caused by pulmonary disease were identified from patient records. Four dogs were excluded: 1 because of documented dehydration on physical examination, 1 because of increased serum creatinine concentration, and 2 because of reported erythrocyte loss, leaving 47 dogs with chronic hypoxemia that were included in the study. The demographic factors of included dogs are presented in Table 1. Median duration of hypoxemia was 6 months (IQR, 3-17 months; range, 1-77 months). The group consisted of 22 WHWTs with CIPF (19 confirmed either by CT or histopathology or both and 3 suspected based on clinical examination, repeated thoracic radiograph findings and severity of hypoxemia) and 25 dogs of other breeds with the following lung diseases: chronic bronchitis (n = 13), unspecified interstitial lung disease (n = 3), and eosinophilic bronchopneumopathy (n = 2). In 7 dogs with chronic respiratory signs

TABLE 1 Demographic information of dogs with chronic hypoxemic (partial pressure of arterial oxygen [PaO_2] < 80 mm Hg) and chronic nonhypoxemic (PaO_2 ≥ 90 mm Hg) pulmonary disease and healthy control dogs

		Hypoxemic dogs	Nonhypoxemic dogs	Healthy control dogs
Number of dogs		47	27	60
Gender	Male	24 (51%)	15 (56%)	32 (53%)
	Female	23 (49%)	12 (44%)	28 (47%)
Weight (kg)		9.5 (8.3-11.4)	14.8 (8.8-25.0)	36.3 (30.1-45.4)[a]
Age (years)		12 (10.0-13.3)[a]	5.3 (3.4-9.0)	4.7 (3.0-7.0)

Note: The data for weight and age are presented as median and interquartile range.
[a]Statistically significant difference ($P < .05$) compared to other groups.

(cough, tachypnea or both) and chronic diffuse radiographic lung changes, a final diagnosis was not established because of lack of advanced examinations in these dogs.

Twenty-seven dogs with nonhypoxemic chronic pulmonary disease (chronic bronchitis, 13/27; eosinophilic bronchopneumopathy, 7/27; mixed bronchial inflammation, 7/27) were included in the diseased comparison group. Median duration of clinical signs was 13 months (IQR, 7-24 months; range, 1-79 months). A control group of 60 healthy dogs consisted of 10 WHWTs and 50 dogs of other breeds. Breed information for all dogs is presented in Table S1, Supporting Information.

3.2 | Clinical examinations

The following respiratory diagnostic tests were performed in patients with hypoxemic pulmonary disease: thoracic radiographs (n = 47/47), bronchoscopy and BAL (n = 31/47), thoracic CT (n = 23/47), lung histopathology (n = 21/47), echocardiography (n = 18/47), and fecal parasitology (n = 40/47). Detailed information on diagnostic examinations is presented in Table S2. The results of the clinical and respiratory examinations are presented in Tables S3 and S4.

Hypoxemia was classified as mild (PaO_2 70-79 mm Hg) in 10 dogs (21.3%), moderate (PaO_2 60-69 mm Hg) in 19 dogs (40.4%), and severe (PaO_2 < 60 mm Hg) in 18 dogs (38.3%). Arterial blood gas measurements at the end of the hypoxemic period (the last visit at which arterial blood gas analysis was performed) for dogs diagnosed with CIPF and other chronic pulmonary diseases as well as arterial blood gas measurements for nonhypoxemic dogs with chronic pulmonary disease and for healthy WHWTs are presented in Table 2.

3.3 | Hematology analysis

Red blood cell parameters measured at the end of the hypoxemia period were within the laboratory reference range for the majority of dogs: Hct was increased in 4/47 (8.5%; 95%CI, 0-17) dogs, Erytr was increased in 12/47 (26%; 95%CI, 13-38) dogs and Hb was increased in 3/47 (6.4%; 95%CI, 0-14) dogs. No marked polycythemia (Hct ≥65%) was noted in any of the dogs. Hematology parameters did not differ significantly between hypoxemic and nonhypoxemic patients (Erytr, $P = .08$; Hb, $P = .24$; Hct, $P = .44$). Red blood cell parameters and laboratory reference ranges are presented in Table 3.

TABLE 2 Arterial blood gas measurements in dogs with chronic hypoxemic (PaO_2 < 80 mm Hg) pulmonary diseases (canine idiopathic pulmonary fibrosis [CIPF] and other chronic pulmonary diseases) and in chronic nonhypoxemic (PaO_2 ≥ 90 mm Hg) pulmonary diseases and in healthy control dogs

	n	PaO_2 (mm Hg)	A-aDO_2 (mm Hg)	$PaCO_2$ (mm Hg)
Hypoxemic dogs with CIPF	22	57.2 ± 8.3[a]	60.6 ± 13.6[a]	29.3 ± 6.5
Hypoxemic dogs with other pulmonary diseases	25	67.6 ± 8.3[b]	41.8 ± 10.2[b]	33.3 ± 3.8
Nonhypoxemic dogs with chronic pulmonary disease	27	95.7 ± 4.8	19.0 ± 5.2	29.5 ± 3.3
Healthy control dogs	10	98.8 ± 7.0	15.8 ± 5.4	29.6 ± 4.3

Note: Measurements were recorded in chronically hypoxemic dogs at the end of the follow up period (mean ± SD) and in nonhypoxemic and healthy dogs at presentation. Arterial blood was sampled at the room air with 21% fraction of inspired oxygen (FiO_2). The data are presented as mean ± SD.
Abbreviations: A-aDO_2, alveolar-arterial oxygen gradient; PaO_2, partial pressure of arterial oxygen; $PaCO_2$, partial pressure of arterial carbon dioxide.
[a]Significantly different compared to hypoxemic dogs with other pulmonary diseases and to nonhypoxemic groups (P < .01).
[b]Significantly different compared to hypoxemic dogs with CIPF and to nonhypoxemic groups (P < .01).

TABLE 3 Red blood cell parameters presented as mean ± SD in dogs with chronic hypoxemic (partial pressure of arterial oxygen PaO_2 < 80 mm Hg) pulmonary diseases, in dogs with chronic nonhypoxemic (PaO_2 ≥ 90 mm Hg) pulmonary diseases and in healthy control dogs

Red blood cell parameter	Unit	Laboratory reference range	Hypoxemic dogs with chronic pulmonary diseases (n = 47)	Nonhypoxemic dogs with chronic pulmonary diseases (n = 27)	Healthy controls (n = 60)
Erythrocyte count (Erytr)	10^{12}/L	5.3-8.0	7.5 ± .7	7.4 ± .9	7.2 ± .7
Hemoglobin concentration (Hb)	g/L	140-203	174 ± 17	169 ± 20	169 ± 15
Hematocrit (Hct)	%	38-57	51.3 ± 5.5	49.9 ± 6.1	50.4 ± 4.6
Mean corpuscular volume (MCV)	fL	67-77	68.5 ± 3.3	67.7 ± 3.4	70.2 ± 3.5[a]
Mean corpuscular hemoglobin (MCH)	pg	22-26	23.2 ± 1.4	22.9 ± 1.2	23.5 ± .9
Mean corpuscular hemoglobin concentration (MCHC)	g/L	318-347	340 ± 15	339 ± 8.9	335 ± 13

[a]Statistically significant difference (P < .05) compared to other groups.

In dogs with chronic hypoxemic pulmonary disease, red blood cell parameters were not correlated with the severity of hypoxemia (correlation to PaO_2: Erytr, $r = -.14$, $P = .36$; Hb, $r = -.21$, $P = .15$; Hct, $r = -.14$, $P = .35$; Figure 1). No significant difference was found in red blood cell parameters between CIPF and other chronically hypoxic dogs (Erytr, $P = .87$; Hb, $P = .44$; Hct, $P = .50$; Figure 2). Duration of

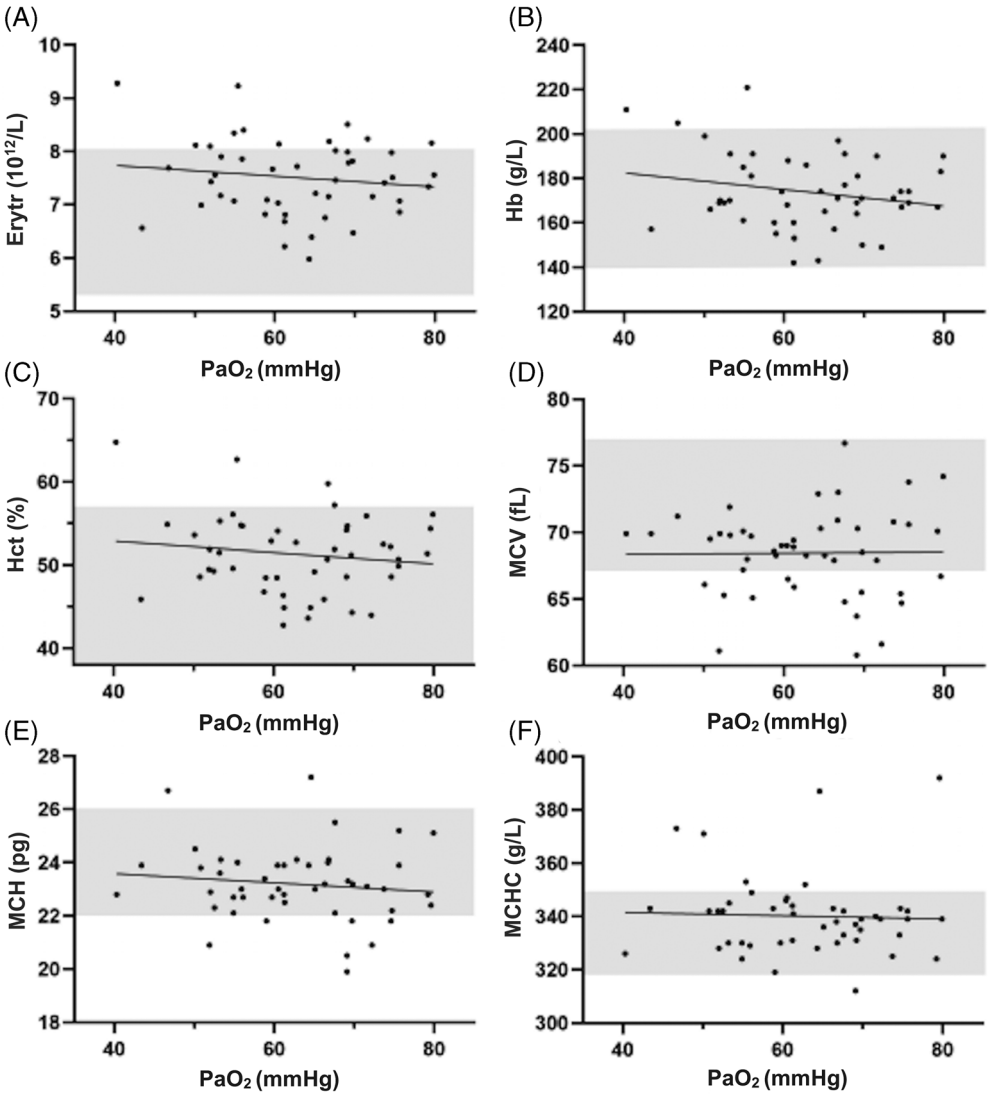

FIGURE 1 Scatter plot representing lack of correlation between erythrocyte count (A), hemoglobin concentration (B), hematocrit (C), mean corpuscular volume (D), mean corpuscular hemoglobin (E), mean corpuscular hemoglobin concentration (F), and partial pressure of arterial oxygen (PaO_2) in dogs with chronic hypoxic pulmonary disease (n = 47). Erytr, erythrocyte count; Hb, hemoglobin; Hct, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration. Laboratory reference range is indicated with gray background color. No statistically significant correlations were detected between red blood cell parameters and PaO_2 ($P > .05$ for all)

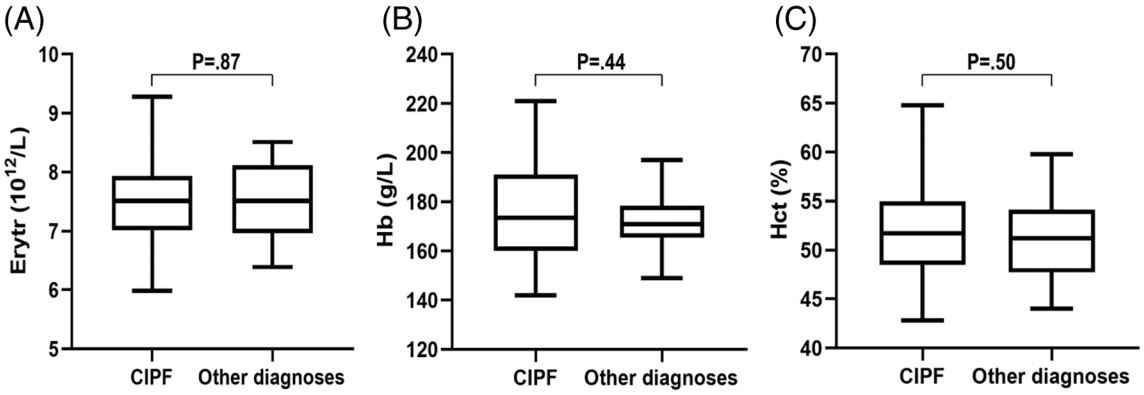

FIGURE 2 Box plots comparing erythrocyte count (A), hemoglobin (B), and hematocrit (C) in dogs with canine idiopathic pulmonary fibrosis (CIPF, n = 22) and in dogs with other chronic pulmonary diseases (n = 25). Vertical boxes represent 25th and 75th percentiles of measured variables, horizontal lines represent median values, and error bars represent 95% confidence interval

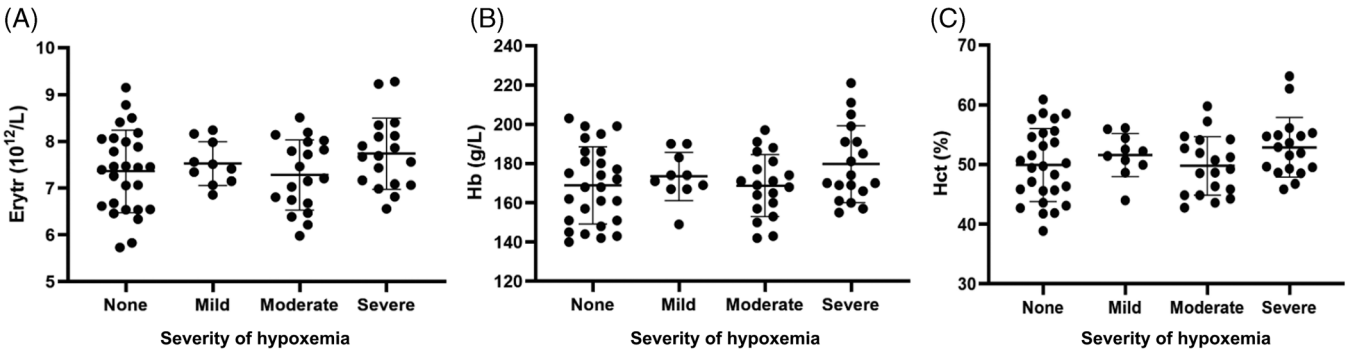

FIGURE 3 A 2D-diagram plotting erythrocyte count (A), hemoglobin (B) and hematocrit (C) in terms of the severity of hypoxemia in dogs with chronic pulmonary disease (n = 74). The dogs with no hypoxemia had partial pressure of arterial oxygen (PaO_2) ≥ 90 mm Hg. Hypoxemia was graded as mild with values PaO_2 70-79 mm Hg, moderate with values PaO_2 60-69 mm Hg and severe with values PaO_2 < 60 mm Hg. The horizontal bar represents mean, the error bars represent SD, and dots represent individual values. There were no significant differences in red blood cell parameters between different severity groups of hypoxemia (P > .05)

the hypoxia was not associated with red blood cell parameters (Erytr B = .001 [95%CI, −.014 to .017], P = .87; Hb B = .005 [95%CI, −.36 to .37], P = .98; Hct B = −.004 [95%CI, −.11 to .10], P = .94). No significant difference was found between red blood cell parameters in the severity groups of hypoxemia in all pulmonary patients (Erytr, P = .29; Hb, P = .19; Hct, P = .23; Figure 3). In the multivariable linear regression model in dogs with arterial blood gas analysis available (n = 84), no association was found in PaO_2 to red blood cell parameters (Erytr B = −.008 [95%CI, −.021 to .005], Hb B = −.21 [95%CI, −.50 to .090], Hct B = −.036 [95%CI, −.13 to .053]; Table S5A). Furthermore, the multivariable linear regression model including all dogs yielded similar results: none of the variables tested was associated with red blood cell parameters (all P-values >.05; Table S5B).

4 | DISCUSSION

In our study, adaptive erythrocytosis was uncommon in dogs with prolonged hypoxemia caused by a chronic pulmonary disease. Erythrocytosis was not associated with the severity or duration of hypoxemia and at most only mild increases in red blood cell parameters were noted. Decreased tissue oxygenation caused by chronic pulmonary disease is mentioned commonly in the veterinary literature as an important mechanism in the development of secondary polycythemia in dogs,[1,2] but our results are contradictory and suggest that chronic pulmonary disease is an unlikely differential diagnosis for dogs presented with severe polycythemia.

Our results support the findings of previous preliminary reports, where no polycythemia was noted in dogs with chronic lung disease. In a cohort of 12 WHWTs with CIPF, no dogs presented with marked polycythemia.[13] Moreover, Hct was normal in 8 dogs with chronic bronchitis and PaO_2 < 80 mm Hg,[10] and in a study of dogs with eosinophilic bronchopneumopathy, no polycythemia was detected in hypoxemic dogs.[24] Herein, we studied the hematology of the hypoxemic dogs with CIPF and other pulmonary diagnoses in a larger cohort with chronic hypoxemia verified by consecutive arterial blood gas analyses. Our study agreed with previous reports in that red blood cell parameters were not significantly increased, despite documented chronic hypoxemia. The observation that erythrocytosis rarely ensues, even from severe hypoxemia in chronically hypoxic pulmonary patients, is in sharp contrast to dogs with cardiac right-to-left shunting, where marked secondary polycythemia occurs because of tissue hypoxemia,[12,25] implying that the underlying mechanisms affecting adaptation and development of erythrocytosis likely are different.

Hypobaric hypoxia at high altitudes is a setting for which both experimental and clinical studies are available in dogs. Acclimatization with compensatory hematopoietic response has been observed in dogs in some studies.[26] However, in more recent studies, exposure to hypoxia occurring at moderately to markedly high altitude resulted in adaptive cardiovascular changes, such as increased heart rate, increased systemic and pulmonary artery pressure, and changes in cardiac function, but not changes in Hct despite mild increases in EPO concentrations.[14,27,28]

In our study, the lack of erythrocytosis in hypoxemic pulmonary disease was similar regardless of the underlying disease process. No statistically significant differences in Hct, Hb, or Eryt were found between dogs with CIPF and those with other chronic lung diseases, even though CIPF dogs were significantly more hypoxemic than dogs with other chronic hypoxic pulmonary diseases. In studies of humans, the type of disease process affects the erythropoietic response to hypoxemia. In human patients, blood Hb and EPO concentrations were lower in IPF when compared to chronic obstructive pulmonary disease (COPD) suggesting a more defective erythropoietic response to hypoxemia in IPF.[19,29] However, although some patients with COPD presented with marked polycythemia, other COPD patients had no or only a mild concurrent increase in red blood cell parameters.[30,31] In our study, because CIPF dogs all were WHWTs, it is impossible to differentiate whether the lack of erythrocytosis was associated with the disease process itself or a breed-related genetic response to hypoxemia.

The cause for the lack of adaptive polycythemia in hypoxic pulmonary patients, both human and canine, remains to be clarified. Lack

of sufficient EPO production could be a possible cause, but in a study of humans with COPD, no significant difference was found in serum EPO between polycythemic and nonpolycythemic groups.[30] This observation could be explained by compensatory suppression of EPO production by increased red blood cell mass in polycythemic patients.[30,32,33] Alternatively, erythropoiesis inhibitors may explain this finding, because several inflammatory cytokines including interleukin-1 (IL-1), IL-2, tumor necrosis factor, interferon-γ, and transforming growth factor-β have been reported to retard the production of erythrocytes.[30,34-37] In humans, defective EPO production and inhibitory effects exerted by pro-inflammatory cytokines have been suspected in IPF.[29] Anemia of chronic disease, as a result of chronic inflammation, is a possible cause, and has been well-characterized in human patients with COPD.[38] In addition, inflammation causing the chronic lung disease also may act to suppress erythropoiesis via impaired iron homeostasis and may shorten erythrocyte survival because of alterations in the erythrocyte membrane.[39] In dogs exposed to hypobaric hypoxia, red blood cells were reported to become less resistant to induced peroxidation, and hemolysis time was significantly decreased.[40] Moreover, other adaptive mechanisms to hypoxia apart from erythropoiesis, such as increases in 2,3-diphosphoglycerate in erythrocytes or genetic changes leading to enhanced Hb-O_2 affinity, as reported to occur in the Tibetan mastiff, may explain the lack of polycythemia.[41-43] Additionally, variation in individual or breed-related genetic responses to hypoxemia may occur in dogs, as differences among human ethnic groups have been reported in the erythropoietic response to EPO treatment or high-altitude exposure.[44,45] Also, because all hypoxemic dogs in our study were relatively old, it cannot be ruled out that old age contributes to the lack of erythropoietic response in these dogs. In order to better understand the pathophysiology behind the lack of compensatory erythropoietic response in dogs with chronic hypoxic pulmonary diseases, additional studies are warranted.

The main limitations of our study included its retrospective design and significant differences in the hypoxemic dogs compared to control dogs (ie, hypoxemic dogs were significantly older than control dogs). Advanced age has been associated with decreases in Hb and red blood cell counts in humans, but it has been concluded that age-specific reference ranges are not needed.[46,47] The effect of advanced age has not been as well established in dogs. A previous study evaluated red blood cell parameters between young and old dogs in a small cohort ($n = 28$) and did not detect significant differences.[48] Similarly, in our study age was not significantly associated with red blood cell parameters. Because of a relatively small sample size, however, our study could have been underpowered to detect subtle differences. However, because red blood cell parameters were not associated with age and did not differ significantly between groups, it is unlikely that the older age of hypoxemic dogs introduced clinically relevant bias in our study. As additional limitations, a final diagnosis was not reached in all dogs and although chronic hypoxemia was confirmed by at least 2 arterial blood gas measurements and the dogs had a documented chronic pulmonary disease process, there was no documentation of persistent hypoxia between the examinations. Therefore, there could have been some variation in the level of hypoxemia between visits. The cohort size was relatively small, which also could have affected the results. For example, in larger cohorts dogs with more severe polycythemia than detected in our study could be encountered. The duration of documented hypoxemia was considered adequate, because in previous studies the erythropoietic response to hypoxemia was detected within 30 days in dogs and after 2 weeks in humans.[26,49]

Overall, in our study we observed that persistent systemic hypoxia caused by chronic lung disease does not commonly result in secondary erythrocytosis in dogs and other mechanisms to accommodate decreased tissue oxygenation are likely. In our study, none of the dogs with chronic hypoxia caused by pulmonary disease had marked erythrocytosis. Thus, lack of adaptive polycythemia is frequent in dogs, even with severe hypoxemia.

5 | CONCLUSION

Our results indicate that polycythemia is uncommonly encountered in dogs with chronic hypoxic pulmonary disease and, when encountered, increases in red blood cell parameters usually are mild.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Study protocols were approved by the Ethics Committee for Animal Experimentation at Helsinki University, Finland (statement numbers 5B/2008, 4/2014, 4/2018, and 13/2020) and by the Committee for Experimental Animals of Southern Finland (ESLH-2008-05403/Ym-23, HY 132-05, ESAVI/1005/04.10.03/2011, ESAVI/5794/04.10.03/2011, ESAVI/7383/04.10.07/2013, ESAVI/9116/04.10.07/2014, ESAVI/9184/04.10.07/2014, ESAVI/10906/04.10.07/2017, and ESAVI/29986/2020).

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

ORCID

Saila Holopainen https://orcid.org/0000-0002-2448-7672

Sanna J. Viitanen https://orcid.org/0000-0002-1208-5799

REFERENCES

1. Couto CG. Erythrocytosis. In: Nelson RW, Couto CG, eds. *Small Animal Internal Medicine*. 6th ed. Elsevier Health Sciences: St. Louis, Missouri; 2019:1368-1370.
2. Stokol T. Anemia, erythrocytosis. In: Ettinger SJ, Feldman EC, Côté E, eds. *Textbook of Veterinary Internal Medicine*. 8th ed. Saunders: St. Louis, Missouri; 2017:229-235.
3. Nitsche EK. Erythrocytosis in dogs and cats: diagnosis and management. *Compend Contin Educ Pract Vet*. 2004;26:104-118.
4. Fliser D, Haller H. Erythropoietin and treatment of non-anemic conditions-cardiovascular protection. *Semin Hematol*. 2007;44:212-217.
5. Maiese K, Chong ZZ, Hou J, et al. Erythropoietin and oxidative stress. *Curr Neurovasc Res*. 2008;5:125-142.
6. Marti HH, Wenger RH, Rivas LA, et al. Erythropoietin gene expression in human, monkey and murine brain. *Eur J Neurosci*. 1996;8:666-676.
7. Broudy VC, Lin N, Brice M, Nakamoto B, Papayannopoulou T. Erythropoietin receptor characteristics on primary human erythroid cells. *Blood*. 1991;77:2583-2590.
8. Ikeda E. Cellular response to tissue hypoxia and its involvement in disease progression. *Pathol Int*. 2005;55:603-610.
9. Heikkilä- Laurila HP, Rajamäki MM. Idiopathic pulmonary fibrosis in West Highland white terriers. *Vet Clin North Am Small Anim Pract*. 2014;44(1):129-142.
10. Padrid PA, Hornof WJ, Kurpershoek CJ, Cross CE. Canine chronic bronchitis: a pathophysiologic evaluation of 18 cases. *J Vet Intern Med*. 1990;4(3):172-180.
11. Reinero C. Interstitial lung diseases in dogs and cats part I: the idiopathic interstitial pneumonias. *Vet J*. 2019;243:48-54.
12. Greet V, Bode EF, Dukes-McEwan J, Oliveira P, Connolly DJ, Sargent J. Clinical features and outcome of dogs and cats with bidirectional and continuous right-to-left shunting patent ductus arteriosus. *J Vet Intern Med*. 2021;35(2):780-788.
13. Heikkilä HP, Lappalainen AK, Day MJ, Clercx C, Rajamäki MM. Clinical, bronchoscopic, histopathologic, diagnostic imaging, and arterial oxygenation findings in West Highland white terriers with idiopathic pulmonary fibrosis. *J Vet Intern Med*. 2011;25(3):433-439.
14. Glaus TM, Hässig M, Baumgartner C, Reusch CE. Pulmonary hypertension induced in dogs by hypoxia at different high-altitude levels. *Vet Res Commun*. 2003;27(8):661-670.
15. Perez-Padilla R, Salas J, Carrillo G, Selman M, Chapela R. Prevalence of high hematocrits in patients with interstitial lung disease in Mexico City. *Chest*. 1992;101(6):1691-1693.
16. Rodriguez FA, Casas H, Casas M, et al. Intermittent hypobaric hypoxia stimulates erythropoiesis and improves aerobic capacity. *Med Sci Sports Exerc*. 1999;31:264-268.
17. Savourey G, Garcia N, Besnard Y, Hanniquet AM, Fine MO, Bittel J. Physiological changes induced by pre-adaptation to high altitude. *Eur J Appl Physiol*. 1994;69:221-227.
18. Savourey G, Garcia N, Besnard Y, Guinet A, Hanniquet AM, Bittel J. Pre-adaptation, adaptation and de-adaptation to high altitude in humans: cardio-ventilatory and haematological changes Eur. *J Appl Physiol*. 1996;73:529-535.
19. Tassiopoulos S, Kontos A, Konstantopoulos K, et al. Erythropoietic response to hypoxaemia in diffuse idiopathic pulmonary fibrosis, as opposed to chronic obstructive pulmonary disease. *Respir Med*. 2001;95(6):471-475.
20. Holopainen S, Rautala E, Lilja-Maula L, Lohi H, Rajamäki MM, Lappalainen AK. Thoracic high resolution CT using the modified VetMousetrap™ device is a feasible method for diagnosing canine idiopathic pulmonary fibrosis in awake West Highland white terriers. *Vet Radiol Ultrasound*. 2019;60(5):525-532.
21. Uhríková I, Lačňáková A, Tandlerova K, et al. Haematological and biochemical variations among eight sighthound breeds. *Aust Vet J*. 1993;91(11):452-459.
22. Määttä OM, Laurila HP, Holopainen S, et al. Reflux aspiration in lungs of dogs with respiratory disease and in healthy West Highland white terriers. *J Vet Intern Med*. 2018;32(6):2074-2081.
23. Määttä OM, Laurila HP, Holopainen S, et al. Matrix metalloproteinase-2,-7, and-9 activities in dogs with idiopathic pulmonary fibrosis compared to healthy dogs and dogs with other respiratory diseases. *J Vet Intern Med*. 2021;35(1):462-471.
24. Rajamäki M, Järvinen AK, Sorsa T, Maisi P. Clinical findings, bronchoalveolar lavage fluid cytology and matrix metalloproteinase-2 and-9 in canine pulmonary eosinophilia. *Vet J*. 2002;163(2):168-181.
25. Moore KW, Stepien RL. Hydroxyurea for treatment of polycythemia secondary to right-to-left shunting patent ductus arteriosus in 4 dogs. *J Vet Intern Med*. 2001;15(4):418-421.
26. Vogel JA, Bishop GW, Genovese RL, Powell TL. Hematology, blood volume, and oxygen transport of dogs exposed to high altitude. *J Appl Physiol*. 1986;24(2):203-210.
27. Glaus TM, Hauser K, Hassig M, Lipp B, Reusch CE. Non-invasive measurement of the cardiovascular effects of chronic hypoxaemia on dogs living at moderately high altitude. *Vet Rec*. 2003;152(26):800-803.
28. Glaus TM, Grenacher B, Koch D, Reiner B, Gassmann M. High altitude training of dogs results in elevated erythropoietin and endothelin-1 serum levels. *Comp Biochem Physiol Part A: Mol Integr Physiol*. 2004;138(3):355-361.
29. Tsantes A, Bonovas S, Tassiopoulos S, et al. A comparative study of the role of erythropoietin in the pathogenesis of deficient erythropoiesis in idiopathic pulmonary fibrosis as opposed to chronic obstructive pulmonary disease. *Med Sci Monit*. 2005;11(4):CR177-CR181.
30. Guidet B, Offenstadt G, Boffa G, et al. Polycythemia in chronic obstructive pulmonary disease: a study of serum and urine erythropoietin and medullary erythroid progenitors. *Chest*. 1987;92(5):867-870.
31. Zhang J, DeMeo DL, Silverman EK, et al. Secondary polycythemia in chronic obstructive pulmonary disease: prevalence and risk factors. *BMC Pulm Med*. 2021;21(1):1-12.
32. Messinezy M, Westwood NB, El-Hemaidi I, et al. Serum erythropoietin values in erythrocytoses and in primary thrombocythaemia. *Br J Haematol*. 2002;117(1):47-53.
33. Messinezy M, Westwood NB, Woodcock SP, Strong RM, Pearson TC. Low serum erythropoietin—a strong diagnostic criterion of primary polycythaemia even at normal haemoglobin levels. *Clin Lab Haematol*. 1995;17(3):217-220.
34. Faquin WC, Schneider TJ, Goldberg MA. Effect of inflammatory cytokines on hypoxia-induced erythropoietin production. *Blood*. 1992;79:1987-1994.
35. Means RT, Krantz SB. Inhibition of human erythroid colony-forming units by tumor necrosis factor requires beta interferon. *J Clin Invest*. 1993;91:416-419.
36. Hansz J, Kozlowska-Skrzypczak M. Interferon-gamma-mediated suppression of erythroid progenitor growth by a HLA-DR and CD4-positive subset of T lymphocytes in acute myeloid leukemia. *Immunobiology*. 1992;186:327-338.
37. Morrone LF, Di Paolo S, Logoluso F, et al. Interference of angiotensin-converting enzyme inhibitors on erythropoiesis in kidney transplant recipients: role of growth factors and cytokines. *Transplantation*. 1997;64(6):913-918.
38. Similowski T, Agusti A, MacNee W, et al. The potential impact of anaemia of chronic disease in COPD. *Eur Respir J*. 2006;27(2):390-396.
39. Fraenkel PG. Anemia of inflammation: a review. *Med Clinics*. 2017;101(2):285-296.

40. Grandjean D, Sergheraert R, Valette JP, Driss F. Biological and nutritional consequences of work at high altitude in search and rescue dogs: the scientific expedition Chiens des Cimes-Licancabur 1996. *J Nutr*. 1998;128(12):2694S-2697S.
41. Finch CA. Erythropoiesis, erythropoietin, and iron. *Blood*. 1982;60(6):1241-1246.
42. Li Q, Liu Z, Li Y, et al. Origin and phylogenetic analysis of Tibetan Mastiff based on the mitochondrial DNA sequence. *J Genet Genomics*. 2008;35:335-340.
43. Signore AV, Yang YZ, Yang QY, et al. Adaptive changes in hemoglobin function in high-altitude Tibetan canids were derived via gene conversion and introgression. *Mol Biol Evol*. 2019;36(10):2227-2237.
44. Gaur P, Saini S, Ray K, et al. Comparative analysis of high altitude hypoxia induced erythropoiesis and iron homeostasis in Indian and Kyrgyz lowlander males. *Curr Res Biotechnol*. 2020;2:120-130.
45. Weisbord SD, Fried LF, Mor MK, et al. Associations of race and ethnicity with anemia management among patients initiating renal replacement therapy. *J Natl Med*. 2007;99(11):1218-1226.
46. Röhrig G, Becker I, Gutensohn K, Nebe T. Red blood cell counts and indices in the elderly German population. *J Lab Med*. 2018;42(4):131-139.
47. Zierk J, Krebs A, Rauh M, et al. Blood counts in adult and elderly individuals: defining the norms over eight decades of life. *Br J Haematol*. 2020;189(4):777-789.
48. Lee SH, Kim JW, Lee BC, Oh HJ. Age-specific variations in hematological and biochemical parameters in middle-and large-sized of dogs. *J Vet Sci*. 2020;21(1):1-13.
49. Garcia N, Hopkins SR, Powell FL. Intermittent vs continuous hypoxia: effects on ventilation and erythropoiesis in humans. *Wild Environ Med*. 2000;11(3):172-179.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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