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Haematological, blood gas and acid-base values in the Galgo Español (Spanish greyhound)

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Objectives: **Haematologic profiles, electrolyte concentrations, blood gas values and acid-base balance have been studied and reported in healthy greyhounds; however, there is only one study published on blood gas values in Galgos Españoles. Because of their purported common origins with greyhounds (same group and class), it was hypothesised that Galgos Españoles also have differences in haematologic values, electrolyte concentrations, blood gas values and acid-base balance compared to other non-sporting breeds.**

Methods: **Venous blood samples from 30 Galgos Españoles and 20 dogs from different breeds were collected, and complete blood counts, electrolyte concentrations, blood gas values and acid-base balance were measured.**

Results: **From the 24 parameters analysed, 5 had statistically significant differences ($P<0{\cdot}05$). Galgos Españoles had higher haematocrit ($P<0{\cdot}001$), haemoglobin concentration ($P=0{\cdot}003$), erythrocyte count ($P=0{\cdot}016$) and pH ($P=0{\cdot}03$), and lower platelet count ($P=0{\cdot}005$), than those in other-breed dogs.**

Clinical Significance: **These results confirm that significant haematologic differences exist in Galgos Españoles when compared with other dogs, although these differences are not as striking as in greyhounds. Practitioners need to be aware of these breed-specific differences in order to make accurate diagnoses in Galgos Españoles.**

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INTRODUCTION

The Galgo Español (Spanish greyhound) is one of the most popular breeds in Spain. Galgos Españoles are mainly used for hunting and live lure coursing, but their importance as pets has increased in recent years, especially after retirement from hunting. Because of its athletic nature and breed classification, the Galgo Español is considered physiologically different from other breeds, and more similar to the greyhound. They are both in group 10 (sighthounds) and section 3 (shorthair sighthounds), according to the Fédération Cynologique Internationale (2011). Haematologic profiles, electrolyte concentrations, blood gas values and acid-base balance have previously been reported in healthy greyhounds (Porter and Canaday 1971, Ilkiw and others 1989, Steiss and others 2000, Shiel and others 2007, Campora and others 2011, Zaldivar-Lopez and others 2011b,c), and several breed-specific peculiarities have been confirmed. As limited information on haematological, electrolyte and blood gas values are available for the Galgo Español (Zaldivar-Lopez and others 2011a), these greyhound-specific peculiarities have simply been extrapolated to the Galgos Españoles in daily clinical practice. Because of the popularity of this breed, especially in Europe, it is important for clinicians to recognise the clinicopathologic peculiarities for veterinary clinical practice.

Greyhounds have higher haematocrit (HCT), haemoglobin (Hb) concentration and red blood cell count (RBC) (Porter and Canaday 1971, Heneghan 1977, Shiel and others 2007, Campora and others 2011), lower total white blood cell count (WBC), neutrophil count and platelet (PLT) count, and atypical eosinophil morphology, when compared with dogs of other breeds (Neuhaus and others 1992, Iazbik and Couto 2005, Shiel and others 2007, Giori and others 2009). Previous studies have also reported differences in blood gas values and acid-base balance in greyhounds compared to other breeds, with a higher Hb affinity for oxygen (Sullivan and others 1994, Zaldivar-Lopez and others 2011c). Recent studies have shown that blood gas values in the Galgos Españoles are also likely outside the reference limits for dogs; they have higher bicarbonate (HCO3) concentration, pCO2, total carbon dioxide (tCO2), total Hb (tHb) content and oxygen content (O2Ct), and lower pH, chloride (Cl) concentration and P50 than mixed-breed dogs (Zaldivar-Lopez and others 2011a).

In Galgos Españoles assessment of CBC, electrolyte concentrations, blood gas values and acid-base balance are of special interest for health screening as part of the blood donor process, because of the increasing use of this breed for this purpose. Some greyhound peculiarities have been demonstrated in other sighthound breeds (Shiel and others 2010), and genetic studies using microsatellite markers have demonstrated that several sighthound breeds cluster together (Parker and others 2007). Therefore, it was hypothesised that the Galgos Españoles, similar to other sighthounds, would also have different haematologic values, electrolyte concentrations, blood gas and acid-base balance values when compared to other non-sighthound breeds. The objective of this study was to evaluate these potential differences between the Galgos Españoles and a group composed of dogs of different breeds.

MATERIALS AND METHODS

Animals

The animals comprised a group of Galgos Españoles and a control group of dogs of other breeds (non-Galgo Español group). All Galgos Españoles were blood donors in an animal blood bank, and the non-Galgo Español dogs were healthy patients (presenting for wellness visits, spays and neuters, and other minor surgical procedures) of the Veterinary Teaching Hospital at the University of Córdoba (Spain). All blood samples were collected after signed informed owner consent and before any other procedures were carried out between February and May 2011. This study was conducted according to European legislation (86/609/EU).

All dogs included in the study had similar lifestyles (i.e., family pets), and were considered to be healthy on the basis of their clinical histories (no illness had been reported in any dog during the preceding year) and physical examination at the time of sample collection. All dogs were negative for five vector-borne diseases (*Leishmania infantum, Ehrlichia canis, Borrelia burgdorferi, Dirofilaria immitis and Anaplasma phagocytophilum*) tested using a commercial ELISA SNAP test (Leishmania Snap and Snap 4Dx, Idexx Laboratories, Barcelona, Spain).

Blood collection procedures

Venous blood samples were obtained by jugular venipuncture using 2-mL plastic syringes and 23 G needles, and blood was placed immediately into 1-mL tubes with ethylenediamine tetraacetic acid (EDTA) for the CBC, and using 1 mL syringes with lithium heparin for electrolyte concentrations, blood gas values and acid-base measurements. All samples were stored in an ice-water bath at 4°C, submitted to the Córdoba University Clinical Pathology Laboratory and analysed between 30 minutes and 2 hours after collection.

Blood analyses

Complete blood counts were performed using an automated haematology impedance analyser (Sysmex F-820), and included determination of RBC, Hb, HCT, mean corpuscular volume (MCV), mean corpuscular Hb (MCH), mean corpuscular Hb concentration (MCHC), WBC, lymphocyte count, granulocyte and monocyte count and PLT count.

Electrolyte concentrations, blood gas values and acid-base balance were measured using the Rapidlab 860 (Siemens Healthcare Diagnostic SL) analyser. This device directly measures pO2, tCO2, pH, sodium (Na+), potassium (K+), ionised calcium (Ca++) and Cl−, and calculates actual and standard HCO3−, tCO2, base excess of blood [BE(B)] and of extracellular fluid [BE(ecf)], estimated oxygen saturation (SO2) and anion gap (AnGap), based on potentiometry and amperometry methods. All analyses were performed following the manufacturer's recommendations and quality controls for assay conditions and instrumentation; a daily maintenance self-test was always done before performing any assay.

Statistical analysis

The dogs were divided into two groups, Galgos Español and non-Galgos Español. The data were analysed using the statistical software SPSS 15.0. Descriptive statistics and a normality test (Kolmogorov-Smirnov test) were performed for all parameters, and both groups were compared statistically using a *t*-test. Levene's test was used to assess the equality of variances. Results for males and females within each group were also compared. Statistical significance was accepted at $P<0{\cdot}05$.

RESULTS

The groups comprised 30 Galgos Españoles and 20 control dogs of other breeds (non-Galgo Español group). The non-Galgo Español group included a variety of large and small breeds (seven mongrels, four beagles, two Labrador retrievers, two bulldogs,

Table 1. Results of haematology measured in 30 Galgos Españoles and 20 other-breed dogs

Parameter	GALGO		Other breeds		P
	Mean	SD	Mean	SD	
RBC ($\times10^{12}$/L)	7·63	0·94	7·01	0·71	0·01
Hb (g/L)	183·63	19·88	169·00	12·90	<0·01
HCT (L/L)	0·52	0·05	0·47	0·03	<0·01
MCV (fL)	68·50	5·56	67·49	5·19	0·52
MCH (pg)	24·15	1·59	24·22	2·18	0·89
MCHC (g/L)	353·2	18·0	358·9	16·6	0·27
WBC ($\times10^{9}$/L)	10·39	4·61	10·38	3·92	0·99
Monocyte/granulocyte count ($\times10^{9}$/L)	8·17	3·92	7·60	3·91	0·62
Lymphocyte count ($\times10^{9}$/L)	2·19	1·05	2·71	1·36	0·14
PLT ($\times10^{9}$/L)	200·79	62·23	267·15	92·84	0·01

RBC Erythrocyte count, Hb Haemoglobin concentration, HCT Hematocrit, MCV Mean corpuscular volume, MCH Mean corpuscular haemoglobin, MCHC Mean corpuscular haemoglobin concentration, WBC Total leukocyte count, PLT Platelet count

Table 2. Results of blood gases, acid-base balance and electrolytes measured in 30 Galgos Españoles and 20 other-breed dogs

Parameter	GALGO		Other breeds		P
	Mean	SD	Mean	SD	
pH	7·34	0·03	7·32	0·04	0·03
pCO_2 (mmHg)	48·03	6·56	49·97	7·31	0·33
pO_2 (mmHg)	30·79	7·96	32·50	10·16	0·51
HCO_3-real (mmol/L)	25·49	3·48	25·10	3·35	0·69
HCO_3-std (mmol/L)	22·78	2·50	22·05	2·56	0·32
CO_2t (mmol/L)	26·97	3·65	26·50	3·48	0·65
BE (B) (mmol/L)	−0·58	2·85	−1·71	2·93	0·18
BE (ecf) (mmol/L)	−0·23	3·69	−1·10	3·54	0·41
SO_2 (%)	52·79	14·81	54·71	19·83	0·69
Ca (mmol/L)	1·11	0·11	1·07	0·09	0·18
K (mmol/L)	4·28	1·09	3·94	0·31	0·18
Na (mmol/L)	137·74	5·77	136·65	4·56	0·48
Cl (mmol/L)	106·81	4·31	106·00	4·91	0·54
AnGap (mmol/L)	10·05	5·84	9·58	5·24	0·77

HCO_3 Bicarbonate, CO_2t Total carbon dioxide, BE(b) Actual base excess, BE(ecf) Base excess in extracellular fluid, SO_2 Oxygen saturation, Ca Ionised calcium, K Potassium, Na Sodium, Cl Chloride, AnGap Anion gap

two fox terriers, one German shorthair pointer, one Podenco and one cocker spaniel). The Galgo Español group included 18 males (60%) and 12 females (40%), with a mean age of 4·7 years (range 1 to 12 years), and a mean weight of 26 kg (range 22 to 30 kg). The non-Galgo Español group included 11 males (55%) and 9 females (45%), with a mean age of 5·2 years (range 1 to 10 years), and a mean weight of 19 kg (range 12 to 27 kg). There were no significant differences between males and females within each group for any parameter. Because of the limited number of neutered and spayed dogs (three males and three females from Galgo Español group, and three males and two females from non-Galgo Español group), this subpopulation was not evaluated in this study.

All the data were normally distributed. Results for CBC are shown in Table 1. From the 10 parameters measured, 4 were statistically significantly different between the groups. Galgos Españoles had higher HCT ($P<0·001$), Hb concentration ($P=0·003$) and RBC ($P=0·016$), and lower PLT count ($P=0·005$) than those in the non-Galgo Español group. There were no statistical differences between the groups for the remaining parameters (MCV, MCH, MCHC, WBC, lymphocyte count, granulocyte and monocyte count).

Results of blood gas values, electrolyte concentrations and acid-base balance are shown in Table 2. From the 14 parameters measured, only the pH was significantly different ($P=0·03$) between the two groups. The Galgo Español group had higher pH ($P=0·03$); however, the remaining parameters (pO2, pCO2, Na+, K+, Ca++, Cl−, actual and standard HCO3−, tCO2, BE(B), BE(ecf), SO2 and AnGap) were not statistically different between the two groups.

DISCUSSION

As published data are limited, reference values for CBC, electrolyte concentrations, blood gas values and acid-base balance in Galgos Españoles have been traditionally extrapolated from greyhounds because of their similarities. In the present study, CBC, electrolyte concentrations, blood gas values and acid-base balance were analysed in a Galgo Español group and in a non-Galgo Español group (that included a variety of different dog breeds) and were compared to assess any differences

The Galgos Españoles had higher HCT, Hb concentration and RBC and lower PLT count than dogs of other breeds, suggesting a real physiological characteristic of this breed, being in agreement with previous reports of greyhounds. Studies of young healthy pretrained and adult greyhounds (retired from racing) have shown that this breed has a higher HCT, Hb concentration and RBC, and a tendency towards lower PLT count when compared with other breeds (Porter and Canaday 1971, Heneghan 1977, Shiel and others 2007, Campora and others 2011, Zaldivar-Lopez and others 2011b).

The CBC results in this study are in general agreement with previous studies in greyhounds, with two exceptions. First, Shiel and others (2007), Campora and others (2011) and Porter and Canaday (1971) showed a lower WBC, lymphocyte and neutrophil count in greyhounds when compared to other breeds. However, in the present study, the total WBC, lymphocyte, granulocyte and monocyte counts were not different in the Galgo Español group when compared with the non-Galgo Español group, suggesting that Galgos Españoles have similar WBC counts to dogs of other breeds. Secondly, macrocytosis has been reported in greyhounds (Porter and Canaday 1971); however, recent studies have shown that their MCVs fall within the reference interval for dogs (Shiel and others 2007, Zaldivar-Lopez and others 2011b). Macrocytosis was first attributed to a shorter erythrocyte lifespan and a higher proportion of immature cells in greyhounds (Novinger and others 1996), but a different study using the same technique has shown no difference in erythrocyte lifespan between greyhounds and non-greyhounds (Garon and others 2010), so further studies are needed to clarify this controversy. There were no differences in MCV between the Galgo Español group and the control population in the present study.

The lower PLT count found in the present study in Galgos Españoles has also been reported in greyhounds, which had PLT counts below established reference intervals for non-greyhound breeds (Sullivan and others 1994, Steiss and others 2000, Shiel and others 2007, Campora and others 2011). The results of this study suggest that a tendency towards a lower PLT count may also be present in healthy Galgos Españoles, and diagnosis of thrombocytopenia should be interpreted with caution in this breed. McDonald and Sullivan (1993) and Sullivan and others (1994) suggested that the stem-cell competition model of haematopoiesis was a possible explanation for the low PLT count in greyhounds (the left shift of the oxygen-Hb dissociation curve in greyhounds could result in mild, chronic hypoxia, leading to increased production of erythropoietin and increased erythropoiesis at the expense of megakaryocytopoiesis). Studies using flow cytometry have discarded immune-mediated disease as the cause of this low number of PLT in greyhounds (Santoro and others 2007). Thrombocytopenia is a common acquired disorder in dogs that can occur as the result of abnormal PLT distribution, decreased PLT production or decreased PLT survival; knowing that Galgos Españoles have a lower PLT count physiologically can help avoid overdiagnosing thrombocytopenia in this breed.

A major limitation of this study is that blood smears were not evaluated, so the results obtained from the analyser were not correlated to those from manual counting methods. Although results obtained with impedance analysers have been shown to correlate well with the manual counting method (Pastor and others 1997), and a daily maintenance self-test was always done before performing any assays, PLT clumps can be recounted as leukocytes or erythrocytes (Pastor and others 1997). Furthermore, this analyser does not differentiate between granulocytes and monocytes, so the differential white cell count should be interpreted with caution.

Another possible limitation of the present study is the high variability in the ages of our dogs (1 to 12 years). However, adult haematology values are reached by approximately 9 to 10 months in young pretraining greyhounds (Shiel and others 2007), and all dogs of our study were more than 1 year of age. Despite the variability induced by the use of different instrumentation, sampling criteria or demographics, and based on the results in Galgos Españoles, it is proposed that these values constitute idiosyncratic physiological characteristics in sighthounds.

The Galgo Español group had a higher pH when compared with the non-Galgo Español group (Table 2). The pH is a clinically relevant value used to ascertain acid-base status, which can result from several ventilatory, metabolic, renal or gastrointestinal conditions. Although the average pH at the time of sampling was higher in Galgos Españoles than in non-Galgos Españoles, this is unlikely to be clinically significant since no significant differences were observed in pCO2, HCO3–, BE, AnGap and strong ion difference [there were no significant differences in free water effect (serum Na+ concentration) and serum Cl– concentration]. Total weak acids (Atot), primarily composed of albumin and phosphorus, were not measured in this study. However, in a study examining serum protein electrophoresis in retired racing greyhounds (Fayos and others 2005), there was no significant difference in albumin compared to non-greyhound dogs, and hypoalbuminaemia, which can produce alkalosis, is unlikely to be clinically significant in Galgos Españoles (unpublished data). Hyperphosphataemia is an important cause of acidosis; however, because serum phosphorus concentration is normally low, hypophosphataemia does not cause clinically significant alkalosis (Hopper and Haskins 2008) and it is unlikely to be the cause of increased pH in Galgos Españoles. Other relevant contributors to the metabolic acid-base component are lactate and unmeasured acids (ketoacids, sulfuric acid, ethylene glycol, salicylic acid, propylene glycol, metaldehyde, D-lactate and ethanol); an increase in these components leads to a decrease in pH (Hopper and Haskins 2008); however, an absence of these components does not cause alkalosis. Hyperventilation caused by anxiety could potentially explain the acid-base status in Galgos Españoles in this study, which would result in a pH increase; however, this is unlikely because the pCO2 was not significantly lower in Galgos Españoles than in other breeds. Neither causes of hyperventilation, such as hypotension, shock, sepsis, exercise, pulmonary parenchymal disease, severe anaemia or pain, nor other causes of increase in pH, such as loss of hydrogen ions by vomiting, drugs or hypokalaemia, were observed in Galgos Españoles of this study.

The results presented here are different from that of a previous study in Galgos Españoles, which reported higher HCO3–, pCO2, tCO2, tHb, O2Ct, and lower pH, serum Cl– concentration and P50, than a mixed-breed group (Zaldivar-Lopez and others 2011a). This could be due to a variety of factors, such as variations in the sampling and storage of blood samples, variations in the analysers used [Vetstat, IDEXX Laboratories (Zaldivar-Lopez and others 2011a) or Rapidlab 860, Siemens Healthcare Diagnostic SL (present study)] or variations in the control group. In this previous study, the control group had only true mixed-breed dogs (Zaldivar-Lopez and others 2011a), while in the present study the control group had pure breed dogs and only seven mixed-breed dogs. Storage of blood samples in ice water to minimise in vitro metabolic changes was performed and samples were evaluated within 30 minutes and 2 hours of the sampling. In a study of the effects of ice-water storage on blood gas and acid-base measurements, Rezende and others (2007) showed that venous pO2 and SO2 were significantly increased from baseline after 4 hours of ice-water storage in 1-mL samples, and pH significantly decreased only after 6 hours of storage. This increase in venous pO2 is attributable to the diffusion of oxygen from and through the plastic of the syringe into the blood, which occurred at a rate that exceeded metabolic consumption of oxygen by the nucleated cells (Rezende and others 2007). No significant changes in pCO2, Hb content, O2t, BE or HCO3– were detected for Rezende in ice-water-stored venous samples that were measured within 6 hours of sampling. All samples in the present study were measured between 30 minutes and 2 hours of sampling, following the same technique previously reported; therefore, it is improbable that the values in the present study were altered by storage. More studies are required to clarify true blood gas values and acid-base balance in Galgos Españoles and the origin of the differences with previous results (Zaldivar-Lopez and others 2011a).

Venous samples were used in this study, instead of arterial blood, based on the National Committee for Clinical Laboratory

Standard (NCCLS) Guidelines, as venous samples are more commonly obtained and practical to evaluate in the clinical setting, and venous blood can provide satisfactory pH and pCO2 values. However, venous pO2 values may not be clinically relevant in a routine clinical study without simultaneous study of arterial pO2 (NCCLS Guidelines). Sample handling could have influenced venous SO2%, pCO2 and pO2 (Wimberley and others 1991); however, all the samples were handled by the same operator (I.M.S.), using the same technique and minimising errors due to room-air contamination by expelling all air from the syringe, capping the syringe, and rolling the syringe between the hands periodically (Ehrmeyer and others 1993). It has been shown that the use of EDTA, citrate, oxalate and fluoride anticoagulants may alter the results of pH, but all our measurements were performed on lithium heparin, which is an acceptable anticoagulant for pH, blood gas values, electrolyte concentrations and metabolite analyses (Ehrmeyer and others 1993).

Blood gas and acid-base balance values have been previously studied in greyhounds. Steiss and others (2000) showed that more than 50% of greyhounds had values outside the canine reference intervals established in their laboratory for Hb, tCO2 and AnGap. The current authors recently reported that greyhounds had significantly higher pH, pO2, SO2, oxyhemoglobin, tHb, O2t, oxygen capacity and oxygen affinity, and significantly lower deoxyhemoglobin when compared with non-greyhound dogs (Zaldivar-Lopez and others 2011c). It is proposed that the high-oxygen-affinity Hb (low P50) found in the greyhound makes greyhound Hb more likely to remain bound to oxygen for longer, therefore being released at a deeper tissue level, where oxygen tension is lower. Sullivan and others (1994) proposed that the lower oxygen release to the tissues could be the reason for the higher RBC, Hb and HCT in greyhounds. However, differences in blood gas values and acid-base balance in the Galgo Español group when compared to the non-Galgo Español group were not found in the present study, with the exception of pH. No significant differences were observed between males and females in this study.

No significant differences in electrolyte concentrations between the Galgo Español group and the non-Galgo Español group were found. To the authors' knowledge, only one study has published results of electrolyte concentrations in Galgos Españoles. Zaldivar-Lopez and others (2011a) reported a lower serum Cl– concentration in Galgos Españoles when compared to mixed-breed dogs; however, in the present study, there were no significant differences for this parameter. Chloride is the major extracellular anion in the body, playing an important role in maintaining electrical neutrality and normal osmolality, and participating in the regulation of acid-base balance; therefore, the true value of this parameter should be established in Galgos Españoles by further studies to avoid misdiagnosis.

Recently, Dunlop and others (2011) established greyhound-specific reference intervals, demonstrating that the Ca++ reference interval is lower in greyhounds when compared to standard reference intervals (Dunlop and others 2011). Steiss and others (2000) reported that greyhounds had higher Na+, K+, Ca++ or Cl– concentrations, compared with the reference intervals of the Auburn University Clinical Pathology Laboratory. Sodium concentrations in greyhounds examined by Porter and Canaday (1971) also were higher than in the mongrel controls. However, no significant differences between the Galgo Español group and the non-Galgo Español group in those parameters were found in this study. Possible confounding factors of environmental and lifestyle in the animals studied could have influenced the results (Ilkiw and others 1989, Rose and Bloomberg 1989, Toll and others 1995, Hill and others 2011) although pet animals with similar physical activity were selected.

A limitation of this study is the small size of the Galgo Español group. A larger study may have identified other significant differences. In addition, it would have allowed the calculation of breed-specific reference intervals in Galgos Españoles, which would have been useful in clinical practice.

CONCLUSION

These results confirm that significant haematologic differences exist in Galgos Españoles, when compared with a group containing dogs of different breeds, although these differences are not as striking as in greyhounds. It can be concluded that Galgos Españoles and greyhounds have similar CBC peculiarities and differences when compared to other dog breeds, but they do not share blood gas, electrolyte concentration and acid-base balance idiosyncrasies previously reported in greyhounds. The present results suggest that some reference intervals (i.e., CBC) should be modified for Galgos Españoles, and veterinary practitioners should be conscious of these differences. CBC, blood gas values, electrolyte concentrations and acid-base balance are commonly evaluated in daily veterinary clinical practice, and Galgos Españoles have many idiosyncrasies that can affect their medical care. Being aware of these will prevent misdiagnoses and will allow more appropriate treatment and diagnostic testing in this breed.

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Conflict of interest
None of the authors of this article has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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