Short Communications

Haptoglobin concentration in galgos and greyhounds

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Greyhounds and galgos Españoles (Spanish greyhounds, GEs) share common origins, and are thus closely related breeds (same breed group and section, according to the Fédération Cynologique Internationale).

Clinicopathological peculiarities of greyhounds have been extensively studied over the last decade. These haematological, haemostatic and biochemical idiosyncrasies have been recently reviewed (Zaldivar-Lopez and others 2011). Despite the similarities between GEs and greyhounds, there are selected phenotypical and physiological differences between them. For example, while greyhounds have a very low frequency of dog erythrocyte antigen 1.1 on the surface of the red blood cells (<15 per cent) (Iazbik and others 2010), GEs have a high frequency (>30 per cent) (Mesa and others 2009).

In 2009, the authors reported that greyhounds have lower serum haptoglobin (Hp) concentrations than non-greyhound dogs; Hp was measured by colorimetric and immunoturbidimetric methods, and confirmed through electrophoresis (Couto and others 2009). Besides systemic immunomodulatory effects (ie, fever, leucocytosis, etc), acute phase response includes changes in the concentrations of acute phase proteins (APPs), which are classified as negative (downregulated) or positive (upregulated). Hp is a positive APP, whose concentration increases rapidly in response to inflammation or tissue injury (Martinez-Subiela and others 2002), in order to remove the noxious stimuli, and restore homeostasis. Hp also acts as a free haemoglobin (Hb) scavenger, preventing tissue oxidative damage and renal dysfunction (Nielsen and others 2010), and has bactericidal effect in infected wounds (by limiting the availability of iron for bacterial growth through Hb-binding) (Murata and others 2004). Hp in dogs is a moderate APP (Conner and others 1988), and changes in concentration have been shown to be of diagnostic and prognostic value in inflammatory processes, such as infectious diseases (leishmaniosis (Martinez-Subiela and others 2002), trypanosomiasis (Ndung'u and others 1991) and after surgical trauma (Ceron and others 2005).

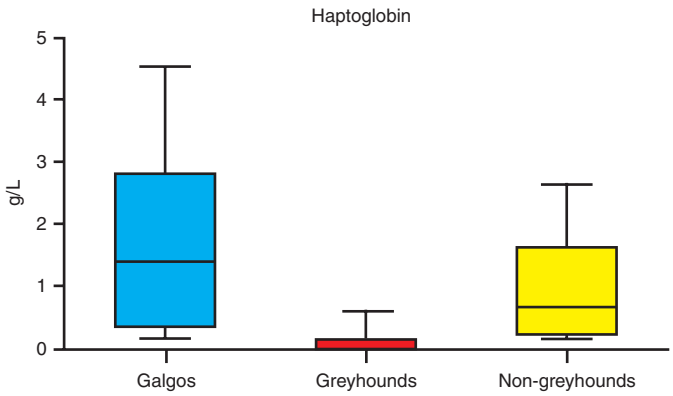

FIG 1: Dot plot graph showing haptoglobin concentration in galgos from this study (mean 1.77 g/l; sd 1.41 g/l). For visual comparison, data (greyhounds and non-greyhounds) from previous publication (Couto and others 2009) were included in the graph. Whiskers represent ranges; therefore, the box contains the central 50 per cent of the data, and whiskers the remaining 50 per cent; the line inside indicates the median

Based on the historical, phenotypical and physiological similarities between the two subbreeds, the authors hypothesised that the low Hp concentration is a common feature of the sighthound group, and thus GEs will have similar Hp concentration to greyhounds. The objective of this study was to measure Hp in GEs, and determine if they are similar to previously reported Hp values in greyhounds.

Venous samples were collected from the jugular vein of 21 healthy adult GEs at the Clinical Veterinary Hospital at the University of Córdoba. Samples were processed within one hour of collection: blood was immediately placed into tubes with EDTA anticoagulant, and centrifuged at 1300 *g* for 10 minutes; plasma was aliquoted into Eppendorf tubes and immediately frozen at −80°C. The following day, all the samples were sent overnight as a batch to the Veterinary Clinical Pathology Laboratory at the Veterinary Hospital at the University of Murcia for analysis. Samples were kept frozen at −80°C until analysis, which was performed three days later. Plasma Hp was measured using a colorimetric Hb-binding method (Tridelta Phase, Tridelta Development). Crossreactivity between the polyclonal goat antihuman Hp antiserum and canine Hp was previously demonstrated by radial immunodiffusion and ELISA tests (Tecles and others 2007). The analysis was performed using a biochemistry autoanalyser (Cobas Mira Plus; ABX Diagnostics), and results were reported in grams per litre (g/l). The same samples were analysed again the following day in order to evaluate the intra-individual variability through coefficient of variation (CV). GraphPad Prism was the software used for statistical analysis. GE data were analysed with descriptive statistics and tested for normality using the D'Agostino & Pearson omnibus normality test.

Since there were two Hp measurements (one day apart), mean values for each dog were calculated, and that was the value used for descriptive statistics. Data in GEs were normally distributed, with a mean of 1.78 g/l (sd, 1.41 g/l), and ranging from 0.13 to 4.52 g/l. Intra-individual CV over the two analyses was very low (8.85 per cent). Raw data (greyhounds and non-greyhounds) from previous publication (Couto and others 2009) were used for the graphical representation (Fig 1).

GEs have plasma Hp concentrations similar to those in other dogs (Ceron and others 2005), in contrast to their closely related sighthound

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groupmates (greyhounds), which have very low or undetectable Hp levels (Couto and others 2009). Surprisingly, GEs have slightly higher Hp than other dog breeds (from the 21 GEs, only four were outside the upper limit of the canine reference interval). This elevation could be breed-specific, or due to a subclinical not previously detected inflammation or infection in some dogs (although all dogs were clinically healthy). Low serum protein concentration in former racing greyhounds (Steiss and others 2000) has been shown to be due to a lower globulin concentration (primarily, serum α- and β-globulin concentrations) (Fayos and others 2005), and the lower Hp concentration likely contributes to the low α-globulin concentrations in the breed (Couto and others 2009). Further studies are warranted in order to investigate the relationship between serum proteins and Hp in GEs.

Hp has two physiological roles: it is a free Hb scavenger, preventing oxidative damage (and consequent hypertension) and renal dysfunction when there is free Hb circulating due to intravascular haemolysis or red blood cell senescence; and it has immunomodulatory effects, since it is an APP. Hp concentration can be used as diagnostic and prognostic marker in various inflammatory processes. Hp concentration in healthy dogs is 0 to 3 g/l (Ceron and others 2005); the magnitude of response to stimuli is a two- to 10-fold increase in plasma concentration occurring in 24 hours, and peaking at three to four days (Ceron and others 2005). A significant increase in serum Hp concentration has been demonstrated after administration of glucocorticoids, anthelmintics and phenobarbital (Martinez-Subiela and others 2004), and a significant positive correlation exists between Hp and the WBC and neutrophil counts (Ndung'u and others 1991).

Hp is more stable than the cellular components of blood; thus, assays can be performed on frozen serum or plasma samples. However, a decrease in Hp concentration in canine serum stored at −20°C has been described; thus, −70°C has been suggested for prolonged storage (Solter and others 1991). In this study, samples were processed immediately, and they were kept at −80°C until analysed to avoid storage changes. Little variation was found between the two measurements of each sample (duplicate measurements, one day apart), as reflected by the low CVs. In dogs with intravascular haemolysis (ie, immune haemolytic anaemia), Hp concentrations are lower because it binds strongly to Hb, and the Hb–Hp complexes are removed from the circulation by macrophages via CD163 (Harvey and West 1987). Considering the previously reported short half-life of greyhound RBCs (Novinger and others 1996), the low Hp concentration could be attributed to chronic haemolysis; however, other indicators of haemolysis (ie, reticulocytosis, hyperbilirubinaemia, increased RDW, etc.) are absent in greyhounds (Zaldivar-Lopez and others 2011), and more recent reports have shown no differences between greyhounds and other breeds' RBC half-life (Garon and others 2010). Although the mechanism of hypohaptoglobinaemia in greyhounds is still unclear, the authors have ongoing molecular studies to investigate if the underlying cause is a decreased Hp gene (*HP*) expression.

Although there is little information in the scientific literature regarding GEs (Weidmeyer and Solter 1996), given the phenotypic similarities between these two sighthound breeds (virtually indistinguishable to the untrained eye), and the difference in Hp concentration, the authors believe that the comparison between greyhounds and GEs could be an interesting natural biological model system to study the physiology of Hp in the canine species. Further studies including more animals from a variety of origins and lifestyles (greyhounds were all retired racers) could help to better understand the Hp-Hb physiology between greyhounds and GEs, potentially giving new insights and encouraging research on treatments for common Hb-scavenging-dependent diseases in dogs (ie, hypertension, thrombosis or infectious diseases). Characterisation and understanding of these mechanisms in greyhounds will be especially beneficial, since many of their peculiarities or common diseases (ie, high creatinine concentration, thromboembolism, hypertension) could be linked to increased free-Hb oxidative damage to tissues (ie, blood vessels, kidney).

Differences between greyhounds and GEs found in this study are of great importance when interpreting laboratory results, since GEs' laboratory work is often interpreted as if they were greyhounds (due to their similarities). As an example, Hp values within the normal range for a GE Galgo (or any other breed) would be elevated for a greyhound.

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References

CERON, J. J., ECKERSALL, P. D. & MARTÍNEZ-SUBIELA, S. (2005) Acute phase proteins in dogs and cats: current knowledge and future perspectives. *Veterinary Clinical Pathology/American Society for Veterinary Clinical Pathology* **34,** 85-99

CONNER, J. G., ECKERSALL, P. D., FERGUSON, J. & DOUGLAS, T. A. (1988) Acute phase response in the dog following surgical trauma. *Research in Veterinary Science* **45,** 107-110

COUTO, C. G., CERÓN, J. J., PARRA, M. D., MARTÍNEZ-SUBIELA, S., IAZBIK, M. C. & LAZBIK, M. C. (2009) Acute phase protein concentrations in retired racing Greyhounds. *Veterinary Clinical Pathology/American Society for Veterinary Clinical Pathology* **38,** 219-223

FAYOS, M., COUTO, C. G., IAZBIK, M. C. & WELLMAN, M. L. (2005) Serum protein electrophoresis in retired racing Greyhounds. *Veterinary Clinical Pathology/American Society for Veterinary Clinical Pathology* **34,** 397-400

GARON, C. L., COHN, L. A. & SCOTT, M. A. (2010) Erythrocyte survival time in Greyhounds as assessed by use of *in vivo* biotinylation. *American Journal of Veterinary Research* **71,** 1033-1038

HARVEY, J. W. & WEST, C. L. (1987) Prednisone-induced increases in serum alpha-2-globulin and haptoglobin concentrations in dogs. *Veterinary Pathology* **24,** 90-92

IAZBIK, M. C., O'DONNELL, M., MARIN, L., ZALDIVAR, S., HUDSON, D. & COUTO, C. G. (2010) Prevalence of dog erythrocyte antigens in retired racing Greyhounds. *Veterinary Clinical Pathology/American Society for Veterinary Clinical Pathology* **39,** 433-435

MARTÍNEZ-SUBIELA, S., GINEL, P. J. & CERÓN, J. J. (2004) Effects of different glucocorticoid treatments on serum acute phase proteins in dogs. *Veterinary Record* **154,** 814-817

MARTÍNEZ-SUBIELA, S., TECLES, F., ECKERSALL, P. D. & CERÓN, J. J. (2002) Serum concentrations of acute phase proteins in dogs with leishmaniasis. *Veterinary Record* **150,** 241-244

MESA, I., GRANADOS, M. M., DOMÍNGUEZ, J. M., MARTÍN-SUÁREZ, E. M. & GALÁN, A. (2009) Determination of 1.1 DEA in Galgo dogs in the province of Cordoba using Rapidvet-H canine DEA 1.1 (DMS) and quick test DEA 1.1 (Alveida). In V Congreso Andaluz de Veterinarios

MURATA, H., SHIMADA, N. & YOSHIOKA, M. (2004) Current research on acute phase proteins in veterinary diagnosis: an overview. *Veterinary Journal (London, England: 1997)* **168,** 28-40

NDUNG'U, J. M., ECKERSALL, P. D. & JENNINGS, F. W. (1991) Elevation of the concentration of acute phase proteins in dogs infected with Trypanosoma brucei. *Acta Tropica* **49,** 77-86

NIELSEN, M. J., MØLLER, H. J. & MOESTRUP, S. K. (2010) Hemoglobin and heme scavenger receptors. *Antioxidants & Redox Signaling* **12,** 261-273

NOVINGER, M. S., SULLIVAN, P. S. & MCDONALD, T. P. (1996) Determination of the lifespan of erythrocytes from greyhounds, using an *in vitro* biotinylation technique. *American Journal of Veterinary Research* **57,** 739-742

SOLTER, P. F., HOFFMANN, W. E., HUNGERFORD, L. L., SIEGEL, J. P., ST DENIS, S. H. & DORNER, J. L. (1991) Haptoglobin and ceruloplasmin as determinants of inflammation in dogs. *American Journal of Veterinary Research* **52,** 1738-1742

STEISS, J. E., BREWER, W. G., WELLES, E. & WRIGHT, J. C. (2000) Haematologic and serum biochemical reference values in retired greyhounds. *Compendium of Continuing Education in Veterinary Practice* **22,** 243-248

TECLES, F., SUBIELA, S. M., PETRUCCI, G., PANIZO, C. G. & CERÓN, J. J. (2007) Validation of a commercially available human immunoturbidimetric assay for haptoglobin determination in canine serum samples. *Veterinary Research Communications* **31,** 23-36

WEIDMEYER, C. E. & SOLTER, P. F. (1996) Validation of human haptoglobin immunoturbidimetric assay for detection of haptoglobin in equine and canine serum and plasma. *Veterinary Clinical Pathology/American Society for Veterinary Clinical Pathology* **25,** 141-146

ZALDÍVAR-LÓPEZ, S., MARÍN, L. M., IAZBIK, M. C., WESTENDORF-STINGLE, N., HENSLEY, S. & COUTO, C. G. (2011) Clinical pathology of Greyhounds and other sighthounds. *Veterinary Clinical Pathology/American Society for Veterinary Clinical Pathology* **40,** 414-425

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