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Acute phase protein concentrations in dogs with nasal disease

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The concentrations of C-reactive protein (CRP), serum amyloid A, haptoglobin (Hp) and α1-acid glycoprotein were measured in dogs with clinical signs of nasal disease and compared with those of healthy dogs in order to determine the expression of these proteins in cases of canine nasal disease. A significant difference (P<0.001) between the symptomatic group and the control group was found for both CRP and Hp. Among the animals with nasal disease, a significant intergroup difference (P<0.05) was found in the expression of Hp between dogs with aspergillosis and those with chronic rhinitis.

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ACUTE PHASE proteins (APPs) are serum proteins whose concentrations change as part of an innate host defence mechanism called the acute phase response. The acute phase response to inflammation protects the host from disease and injury, minimises tissue damage and enhances the rate of repair (Eckersall 2000). The acute phase response is stimulated by the release of proinflammatory cytokines including interleukin-1, tumour necrosis factor-α and interleukin-6 from monocytes and macrophages in response to tissue damage or infection (Dinarello 1984, Heinrich and others 1990). Positive APPs, which include C-reactive protein (CRP), serum amyloid A (SAA), haptoglobin (Hp), α1-acid glycoprotein (AGP) and caeruloplasmin, all show increased plasma concentrations after tissue injury, whereas both albumin and transferrin decrease in concentration and are described as negative APPs. Both CRP and SAA are considered to be major APPs because their concentrations in plasma are characterised by an early sharp rise followed by a rapid decline. Hp and AGP are moderate APPs: they show more gradual increases of smaller magnitude, and then return to normal reference levels.

CRP, the first APP to be discovered, has a molecular weight of 100 kDa. Higher mortalities have been associated with elevations in plasma concentrations of CRP in cases of aortic aneurysm in human beings (Schillinger and others 2002). CRP has also been shown to be a sensitive and inexpensive marker of inflammation for the early diagnosis of bacterial infection after orthopaedic surgery in human beings (Waleczek and others 1991). In dogs, CRP has been shown to be elevated in various disorders, including pancreatitis, pyometra, pneumonia, immune-mediated haemolytic anaemia and postsurgical trauma (Yamamoto and others 1993, 1994, Fransson and others 2004, Holm and others 2004, Couto and others 2009, Mitchell and others 2009). A decrease in the level of CRP has also been shown to be correlated with survival in dogs with systemic inflammatory response syndrome or sepsis (Gebhardt and others 2009).

SAA is a small serum protein with a molecular weight of 11,685 Da. It is produced by hepatocytes and is a non-specific marker of infectious, inflammatory, immunological and traumatic disease (Eckersall 1995). The plasma concentrations of SAA have been shown to increase more rapidly compared with other APPs; in dogs, horses and human beings the plasma concentrations may increase 100- to 1000-fold in response to acute inflammation (Pepys and others 1989, Eckersall and others 1999a, Glojnic and others 2001).

Hp is a glycoprotein that is synthesised in the liver. Its primary role is in the complexing of free haemoglobin after intravascular haemolysis. Serum concentrations of Hp decrease in cases of haemolytic disease and increase in response to inflammatory disease (Mischke and others 2007).

Canine AGP, as in other species, is a protein with an unusually high proportion of glycosyl groups (45 per cent) contributing to its molecular weight of 45 kDa. Changes in glycosylation of AGP have been described in human beings and also in cats with feline infectious peritonitis (Ceciliani and others 2004).

Canine nasal disease is a common clinical entity seen in small animal practice, which can arise due to neoplasia, foreign bodies, inflammation, bacterial, mycotic or parasitic infections and dental disease (Tasker and others 1999, Meier and others 2008). The aetiology of nasal disease in dogs remains a diagnostic challenge, and a definitive diagnosis cannot be made from clinical findings alone. Diagnosis of
canine nasal disease usually requires a combination of investigative techniques including radiography, CT, MRI, rhinocytology, bacteriology, mycology and cystological or histological examination of nasal samples collected at the time of investigation. A recent retrospective study showed that a definitive diagnosis could not be established in 36.5 per cent of cases of canine nasal disease (Meler and others 2005).

The purpose of this study was to determine whether serum concentrations of APPs alter in clinical cases of canine nasal disease, and to investigate whether there is a significant variation in plasma APP concentrations among the different phenotypic groups diagnosed.

Materials and methods
Client-owned dogs presented to the authors’ institutions between September 2007 and September 2009 with a history of persistent nasal disease were considered eligible for inclusion in the study. All the cases received a full clinical examination with specific emphasis on nasal examination. This included examination of the external nares for evidence of discharge and the nature of the discharge, if any; examining for evidence of nasal planum ulceration or depigmentation; visual examination and palpation for the presence of facial asymmetry and/or pain; assessment of nasal patency; and palpation of regional lymph nodes for evidence of enlargement.

Blood samples were collected at the time of presentation and before any invasive procedures, for routine haematology, biochemistry and aspergillosis serology tests and an APP panel. The panel of APPs run for this study was measured on residual samples taken for a primary clinical purpose, which would have otherwise been discarded as clinical waste. The panel comprised CRP, SAA, AGP, and Hp. Serum samples taken for analysis of the APPs were stored at −20°C until assayed. The concentrations of the APPs were determined as described previously by Lowrie and others (2009a, b). The CRP immunoturbidimetric assay and Hp haemoglobin binding capacity assays have been described elsewhere by Eckerrall and others (1991, 1999b). SAA was measured using a commercial canine ELISA kit (Tridelta Development), and AGP was measured using a commercial radial immunodiffusion assay (I-Path) in accordance with the manufacturer’s instructions. The limit of detection of the CRP immunoassay, based on 2 sd from blank samples with zero or negligible amounts of CRP, was 0.73 mg/l, and had interassay coefficients of variation (CVs) (n=20) of 24 and 24 per cent with control samples of 39 and 140 mg/l, respectively. For SAA, the limit of detection was 0.12 mg/l with interassay CVs (n=9) of 31 and 12 per cent with control samples of 9.3 and 50 mg/l, respectively. Although these CVs were higher than for clinical chemistry analytes, they were considered acceptable for immunoassay because they were all less than the recommended 25 per cent (Fendick and others 2000) except for SAA at a low concentration (9.3 mg/l, CV=31 per cent). However, given that the serum concentration of this analyte in dogs can rise to over 2000 mg/l (Lowrie and others 2009b) and given the large dynamic range, this higher CV was also considered acceptable. The limit of detection of the Hp assay was 0.02 g/l and interassay CVs (n=18) were 5 and 7 per cent with control samples of 0.3 and 1.0 g/l, respectively. The limit of detection of the AGP assay was 0.02 g/l and a control sample concentration of 0.45 g/l (n=3) had an interassay CV of 2.7 per cent.

The serum APP concentration results from the dogs with nasal disease were compared with those from dogs (irrespective of breed, age or sex) that were free from inflammatory or infectious disease. Serum samples previously described by Mischke and others (2007) were included in the control samples for this study. However, not all samples from healthy dogs from the laboratory’s serum bank were available for all APP assays. The sera were assayed for concentrations of CRP (n=51), Hp (n=54), AGP (n=37) and SAA (n=39), using the methods described above.

Diagnostic imaging procedures were performed for all dogs under sedation or general anaesthesia. These included radiography of the nasal cavities (right or left lateral, intraoral dorsoventral and rostrocaudal view of frontal sinus), CT or MRI.

The nasopharynx and choanae of all the dogs were examined with a retroflexed endoscope using an 8.5 mm flexible videendoscope (Olympus) for evidence of loss of symmetry, inflammation, foreign bodies or masses. The pharynx was packed with gauze swabs and a 30° 9.5 Fr (including sheath) rigid cystoscope (Karl Storz) with a sterile saline flushing channel was used for anterograde rhinoscopy. The dorsal and ventral meatus were inspected for evidence of neoplasia, fungal plaques, turbinate destruction and foreign bodies. Biopsy samples collected during endoscopic examination were submitted for histopathological examination and bacterial and fungal culture.

The dogs were assigned to one of three groups – neoplasia, aspergillosis or chronic rhinitis – based on the diagnostic imaging findings, positive results for either fungal culture or aspergillosis serology and the results of the histological analysis.

The data were explored graphically and statistical comparisons of APP levels among groups (control, aspergillosis, rhinitis and neoplasia) were carried out using Kruskal Wallis tests. Mann Whitney U tests were used for post hoc pairwise comparisons. Significance was set at P<0.05, and multiple comparisons were accounted for using a Bonferroni correction. All analyses were undertaken in R (R Development Core Team 2009).

Dogs were excluded from the study if they had received steroid treatment in the seven days before APP sampling, because steroid treatment is known to stimulate the production of Hp in dogs (Martínez-Suárez and others 2004, Cerón and others 2005).

This study was approved by the welfare and ethics committee of the University of Glasgow.

Results
Forty-eight dogs with nasal disease were included in the study. The animals were assigned to one of three groups depending on their diagnosis: neoplasia (n=13), aspergillosis (n=13) and rhinitis (n=17). The mean (sd) age of the animals in the study was 7.79 (2.82) years (range 0.5 to 12 years). The breeds included cocker spaniel (n=5), golden retriever (n=5), Border collie (n=3), Staffordshire bull terrier (n=3), German shepherd dog (n=3), rottweiler (n=3), West Highland white terrier (n=2), jack Russell terrier (n=2), springer spaniel (n=2), lurcher (n=2), Cavalier King Charles spaniel (n=2), samoyed (n=2) and one each of the following: German pointer, bull mastiff, boxer, labrador retriever, briard, English bulldog, greyhound and German visc. There were also six crossbreed dogs.

The median and range of CRP concentrations in the three groups of dogs with nasal disease are given in Table 1 and shown in Fig 1, along with the concentrations found in healthy dogs (Mischke and others 2007). Post hoc comparisons revealed that serum samples from each of the three groups of dogs with nasal disease had elevated CRP concentrations compared with the samples from healthy animals (P<0.001 for each group relative to controls). However, there were no significant differences in CRP levels among the groups with different nasal diseases.

The median and range of Hp concentrations in the three groups of dogs with nasal disease are given in Table 1 and shown in Fig 2, along with the concentrations found in healthy dogs. Again, post hoc comparisons identified the differences between the serum samples from each of the diseased groups and the samples from healthy animals to be statistically significant (P<0.001 for each group relative to control).
The median Hp concentration in the aspergillosis group (5.2 g/l) was also found to be significantly different (P < 0.01) from the median concentration in the rhinitis group (2.9 g/l).

The median and range of AGP concentrations in the three groups of dogs with nasal disease are given in Table 1 and shown in Fig 3, along with the concentrations found in healthy dogs. There were no significant differences among the groups (P = 0.612) as determined by non-parametric analysis of variance (Kruskal-Wallis test), irrespective of whether the samples from the healthy group were included in the analysis.

The median and range of SAA concentrations in the three groups of dogs with nasal disease are given in Table 1 and shown in Fig 4, along with the concentrations found in healthy dogs. A significant difference (P < 0.05) was found between the neoplasia group and the control group.

**Discussion**

APPs are widely used as non-specific markers of inflammation in dogs (Eckersall 2000, Cerón and others 2005). Recent publications have shown that APPs aid in the diagnosis of certain conditions and are of prognostic value (Conner and others 1988, Holm and others 2004, Gebhardt and others 2009). APPs have also been shown to be elevated in normal physiological conditions such as pregnancy (Ulutas and others 2009). Recent studies in children have shown that CRP is downregulated in allergic rhinitis (Steiner and others 2006).

Canine SAA and CRP have been shown to be the major APPs in dogs (Cerón and others 2005). Changes in CRP and SAA have been shown to occur in pathological conditions such as sepsis (Gebhardt and others 2009), steroid-responsive meningitis-arteritis (SRMA) (Lowrie and others 2009a), and in bitches with pyometra being monitored for postoperative complications (Dabrowski and others 2009).

In the present study, CRP levels were found to be significantly higher in dogs with nasal disease (irrespective of the type of nasal disease) compared with controls (P < 0.001). This was an expected finding, on the basis of results from earlier studies evaluating CRP in dogs with other inflammatory, infectious and neoplastic diseases (Burton and others 1994, Mischke and others 2007, Lowrie and others 2009a), and in bitches with pyometra being monitored for postoperative complications (Dabrowski and others 2009).

The results of the SAA analysis show a significant difference between the neoplasia group (P < 0.05) and the control group (P < 0.05), but not between the aspergillosis group or chronic rhinitis group and the control group. The explanation for the significantly higher relative levels of CRP but not of SAA in the aspergillosis and rhinitis groups compared with the controls is not clear. The findings suggest, however, that the mechanisms that control these major APPs in dogs are different from those in human beings (Cerón and others 2005). It is possible that SAA production is stimulated only under certain conditions or at particular levels of pathogenesis. The results of the present study contrast with those of a study that investigated the APP response to SRMA (Lowrie and others 2009a). It is evident that SRMA caused the stimulation of both APPs, possibly due to differences in the locations of the lesions or to the severity of the inflammatory process. Further investigation is warranted to determine the explanation for this finding, and to interpret the differential elevations.
of these APPs under different conditions with a view to improving the accuracy of diagnosis.

Hp and AGP are APPs that show moderate elevation in levels during acute phase reactions (Cerón and others 2005). The concentrations of Hp and AGP have been shown to increase in many infectious and inflammatory conditions, including surgical trauma (Conner and others 1998). SRMA (Lowrie and others 2009a) and lymphatic neoplasia (Mischke and others 2007). Significant differences in Hp concentrations (P < 0.001) were found between the groups of animals with nasal disease and the control group, but no differences were found in the mean AGP concentrations among the groups. In addition, a significant difference (P < 0.01) was detected between the serum concentrations of Hp in the aspergillosis group and the chronic rhinitis group. Of the moderate APPs in dogs, Hp has been found to be more responsive than AGP in other conditions such as SRMA. A significant increase in serum Hp concentration has also been shown after administration of different dosages of exogenous glucocorticoids (Crener and others 2005). 

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Conflict of interest

PDE is a director and shareholder of ReactivLab, LB is an employee of ReactivLab.

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