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Dogs with persistent disease were readily identified with the profile of postoperative plasma ACTH and cortisol values. Preoperative α-MSH concentrations and postoperative plasma ACTH, cortisol and α-MSH concentrations were prognostic for recurrence of hyperadrenocorticism.

It is concluded that postoperative measurement of plasma concentrations of ACTH, α–MSH and cortisol is easily performed and valuable for early postoperative evaluation of long-term outcome after transsphenoidal hypophysectomy in dogs with PDH.

ABSTRACT #22

URINARY ALDOSTERONE TO CREATININE RATIO BEFORE AND AFTER SUPPRESSION WITH ORAL FLUDROCORTISONE ACETATE IN CATS. S.C. Djajadiningrat-Laanen, S.E. Cammelbeeck, S. Galac, H.S. Kooistra. Department of Clinical Sciences of Companion Animals, Utrecht University, Utrecht, The Netherlands.

The diagnosis of primary hyperaldosteronism in cats is currently based on the ratio between the plasma aldosterone concentration and plasma renin activity, i.e., an elevated aldosterone to renin ratio (ARR). Since the ARR has a number of disadvantages, a more practical diagnostic parameter would be preferable. We therefore aimed: 1. to establish a reference range for the basal urinary aldosterone to creatinine ratio (UACR) in cats; 2. to investigate whether oral fludrocortisone acetate can be used to suppress aldosterone secretion in healthy cats.

Morning urine samples from 42 healthy cats were collected for the determination of the basal UACR. Inclusion criteria were an unremarkable physical and routine laboratory examination, a systemic arterial blood pressure <160 mm Hg, and an ARR below the upper limit of the reference range. Successively, fludrocortisone acetate was administered to 16 healthy cats and one cat with primary hyperaldosteronism, in an oral dosage of 0.05 mg/kg BW BID for four consecutive days. The following morning, urine was collected for the UACR after oral fludrocortisone acetate administration.

Basal UACRs ranged from $1.8–52.3*10^{-9}$ and non-parametric analysis revealed that the reference range for the basal UACR was $<46.5*10^{-9}$. Oral administration of fludrocortisone acetate caused a reduction in UACR of more than 40% and resulted in a UACR $<6*10^{-9}$ in all 16 healthy cats. In the cat with primary hyperaldosteronism, the basal UACR and the UACR after fludrocortisone acetate administration were $32*10^{-9}$ and $36*10^{-9}$, respectively.

The results of this study suggest that determination of the UACR, in combination with a fludrocortisone suppression test, may be used to diagnose primary hyperaldosteronism in cats. Dose finding studies and inclusion of more cats with primary hyperaldosteronism are warranted.

ABSTRACT #23

EVALUATION OF THYROID FUNCTION WITH RECOMBINANT HUMAN THYROID STIMULATING HORMONE AND SCINTIGRAPHY IN HEALTHY CATS. I. van Hoek[1], K. Peremans[2], E. Vandermeulen[2], L. Duchateau[3], S. Daminet[1]. [1]Department of Medicine and Clinical Biology of Small Animals, [2]Department of Medical Imaging of Domestic Animals, [3]Department of Physiology and Biometrics, Faculty of Veterinary Medicine, Ghent University, Belgium.

Studies in humans with nodular goiter have demonstrated that administration of recombinant human Thyroid Stimulating Hormone (rhTSH) increases the uptake of radioiodine in the thyroid. This results in lower therapeutic doses needed and less irradiation to extra-thyroidal tissue (Nieuwlaat, 2003). Because the thyroid gland of healthy euthyroid cats can be stimulated by rhTSH (Stegeman, 2003) we hypothesized there could be a similar application of rhTSH in hyperthyroid cats as in humans. We investigated whether rhTSH could influence uptake of pertechnetate (which is pumped inside the thyroid cell by the same sodium-iodide symporter as iodide) in the thyroid gland of healthy cats.

Six healthy euthyroid female cats, with an age of 2 years, a bodyweight of 4.7±0.4 (mean±SD) kg and showing no abnormalities on clinical examination, blood- and urinalysis, were included. A pertechnetate scan was performed on day 1. Two mCi pertechnetate was injected IV and static images were acquired 30 minutes after injection under anaesthesia (8 mg/kg Propofol IV until effect). Regions of interest (ROI) were drawn manually to calculate the thyroid/salivary gland (T/S) uptake ratio in both thyroid lobes. On day 3 0.025 mg rhTSH (Thyrogen®, Genzyme corporation, the Netherlands) was injected IV. Six hours later the pertechnetate scan was repeated as on day 1. Two bloodsamples were drawn from the jugular vein by venipuncture, before injection of the rhTSH and the pertechnetate scan respectively. Serum was collected after centrifugation, aliquoted and frozen at −20°C until radioactivity had decayed for measurement of total T4 (TT4, nmol/L). Results are expressed as mean±SD. Based on a fixed effects model, serum TT4 concentration increased significantly ($P<0.001$) from 0 hours (19.1±4.6) to 6 hours (54.4±5.9) after rhTSH administration. T/S uptake ratio was analysed by a mixed model with cat and lobe as random effects and rhTSH administration, side (left or right) and their interaction as fixed effects. There was a significant effect of rhTSH administration ($P=0.013$) and of side ($P=0.039$) with a non-significant interaction ($P=0.925$). In the left (right) lobe, T/S uptake ratio increased from 1.12±0.07 (0.97±0.07) to 1.27±0.07 (1.13±0.07) from 0 to 6 hours after rhTSH administration. Pearson correlation coefficient between difference in serum TT4 concentration and T/S uptake ratio before and after rhTSH administration was −0.278 and not significantly different from 0 ($P=0.59$).

Uptake of pertechnetate in the thyroid of euthyroid cats is marginally influenced by 0.025 mg rhTSH 6 hours after administration. Further studies are necessary to optimize pertechnetate uptake by varying time intervals and doses.

ABSTRACT #24

EFFECT OF BREED ON BODY COMPOSITION IN DOGS. I. Jeusette[2], F. Aquino[1], A. Fischetti[1], C. Torre[2], M. Peterson[1], D. Greco[1]. [1]The Animal Medical Center, New York, NY; [2]Affinity-Petcare, Barcelona, Spain.

Recently, breed diversity has been characterised by comparison of genetic material from various breeds of dogs to the wolf (Ostrander et al, 2004). Dual X-ray absorptiometry (DEXA) methodology to estimate body composition has been validated in dogs by comparison with chemical analysis and is considered to be a reference method. The first objective of this clinical study was to assess the effect of breed on percentage body fat mass (measured by DEXA) in dogs. Breeds (Siberian Husky, Greyhound, Standard Poodle, Dachshund, Rottweiler) were chosen based on their relationship to the wolf (asian, herders, steppers, mastiffs). The second objective was to compare results of body fat obtained by DEXA analysis with results obtained by bioelectrical impedance (BIA), and morphometric analysis in these dogs.

Healthy client-owned dogs of selected breeds that were sedated or anaesthetised for unrelated reason were enrolled in the study (n=17, 5 Greyhounds, 3 Standard Poodles, 4 Siberian Huskies, 2 Dachshunds, 3 Rottweilers). Firstly, body weight (BW) was recorded and a body condition score (BCS) was given to each patient according to the 9-point scale (Laflamme et al, 1997), to assess subjectively the degree of leanness/obesity. Then, various morphometric measurements were taken as described by Mawby et al (2004) and used in equations to estimate body fat. Secondly, each anaesthetised patient was submitted to a DEXA scan (Lunar DPXαlpha system, Lunar corp, Madison WI) and to bioelectrical impedance analysis (BIA) (RJL systems, Clinton MI), using electrodes in 3 different positions: on the two left legs (Left hemisphere), on the two right legs (right hemisphere), on the two front legs (front hemisphere). Data were normally distributed and were submitted to a univariate and multivariate analysis of variance and correlations were calculated. Differences were considered statistically significant at $P<0.05$.

In the univariate analysis of variance, DEXA fat mass significantly differed between breeds. For a same mean BCS, Greyhound had significantly less fat then Poodle, Rottweiler, Dachshund and Husky. When including the BCS and breed effects together in the multivariate analysis of variance, the differences between breeds still tend to be significant. In this study, no significant correlation was observed between percentage fat (by DEXA) and BCS, BW, percentage body fat estimated by BIA, or by morphometric equations. BCS correlated with percentage fat (by DEXA) in Greyhound and Poodle but not in Rottweilers or Huskies. Percentage fat (by BIA) correlated with DEXA fat in Huskies and Rottweilers. Body mass index tended to correlate with DEXA fat in large dogs (Greyhound, Husky and Rottweiler) but not in Poodles.

In conclusion, the Greyhound breed is significantly leaner than other breeds of dogs for the same BCS. More data are required but it seems that the current morphometric equations are not adapted to the different breed morphology. Development of breed specific BCS and equations could be envisaged in the future.

ABSTRACT #25

ACUTE PHASE PROTEIN CONCENTRATIONS IN DOGS WITH HYPERCORTISOLISM, DIABETES MELLITUS AND HYPOTHYROIDISM. F. Fracassi, F. Dondi, E. Mercuriali, A. Mazzi, P. Famigli-Bergamini, F. Gentilini. Veterinary Clinical Department, University of Bologna, Italy.

The purpose of this study was to determine the concentrations of Haptoglobin (Hp), C-reactive protein (CRP) and Fibrinogen (Fib) in dogs with spontaneous hypercortisolism (HCT), diabetes mellitus (DM) and hypothyroidism. Stored (−20°C) serum (Hp and CRP) or citrate plasma (Fib) samples from 35 dogs with HCT (31 PDH and 4 FAT), 24 with DM and 7 with hypothyroidism were analyzed. All samples were obtained from newly diagnosed dogs before starting the therapy for the specific endocrinopathy. Reference ranges for APPs were previously obtained from a population of 25 clinically healthy dogs. CRP and Hp were measured using human immunoturbidometric assays validated in our laboratory for the dog, as previously reported. CRP concentrations (reference range 0–0.5 mg/dl) were between 0.01 and 1.63 (median 0.01; abnormal in 14.3% of cases), between 0.01 and 11.60 (median 1.03; abnormal in 62.5% of cases), and between 0.01 and 9.82 (median 0.01; abnormal in 42.9% of cases) in HCT, DM and hypothyroid dogs respectively. Hp concentrations (reference range 20–140 mg/dl) were between 0 and 590 (median 276; abnormal in 82.9% of cases), between 61 and 387 (median 152; abnormal in 62.5% of cases), and between 2 and 242 (median 109; abnormal in 42.9% of cases) in HCT, DM an hypothyroid dogs respectively. Fib concentrations (reference range 1.45–3.85 g/l) were between 0.82 and 6.16 (median 3.77; abnormal in 47.1% of cases), between 2.32 and 4.27 (median 3.63; abnormal in 41.7% of cases), and between 3.31 and 6.69 (median 4.40; abnormal in 71.4% of cases) in HCT, DM an hypothyroid dogs respectively.

CRP concentrations were significantly lower ($p<0.001$) in HCT compared to DM dogs. Hp concentrations were significantly higher in HCT compared to DM ($p<0.001$) and hypothyroidism ($p=0.003$). Fib was higher in dogs with hypothyroidism compared to dogs with DM but not statistically significant ($p=0.051$). Only 5 dogs with HCT had a (mild) increase of CRP: 2 had also a severe pyoderma, 2 concomitant DM with ketoacidosis and 1 concomitant mediastinal tumour and haemolytic anaemia. In these cases, we considered the increase of CRP inadequate when compared to the expected acute phase response. The high serum concentrations of Hp in dogs with HCT has previously been reported and our results are comparable to those reported in the literature. Lack of exact knowledge regarding the inflammatory/infectious status of each dog is the main limitation of this study. In conclusion, APPs were high in a large number of dogs with endocrinopathies and this is probably due to the high incidence of concomitant infectious diseases. CRP is low in dogs with HCT and, like Hp, in this endocrinopathy should be considered a poor marker of the acute phase response. Further studies are required to assess whether serum Hp and CRP could be useful for the diagnostic protocol of dogs with HCT.

ABSTRACT #26

DIFFERENTIAL EXPRESSION OF TOLL LIKE RECEPTOR 2 AND 4 IN DUODENAL BIOPSIES FROM DOGS WITH INFLAMMATORY BOWEL DISEASE PREDICTS SEVERITY OF DISEASE.

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There is increasing evidence that aberrant innate immune responses towards the bacterial flora of the gut play a role in the pathogenesis of canine inflammatory bowel disease (IBD). It is possible that individual differences in severity of disease and response to treatment are due to differential expression of Toll-like receptors (TLRs) or their response to microbial ligands in the intestinal lumen.

The aim of this study was to evaluate TLR2 and TLR4 mRNA expression in duodenal biopsies from dogs with IBD and healthy dogs and to correlate the expression profiles with severity of clinical disease.

Sixteen clinical cases were scored for disease severity using a modification of the canine IBD activity index. RNA was extracted from endoscopic duodenal biopsies from the 16 diseased dogs and 7 healthy control dogs. TLR2 and TLR4 mRNA expression was assessed quantitatively using specific primers and real-time PCR.

TLR2 and TLR4 mRNA expression was not significantly elevated in the IBD dogs as a whole compared to controls. However, the diseased dogs appeared to be divided into two distinct groups: Group 1 (n=6) showed a 2–4 times higher expression of TLR2 and TLR4 compared to controls, whereas group 2 (n=10) showed similar expression of TLR2 and TLR4 compared to healthy control dogs. Group 1 dogs had significantly higher clinical scores (median score of 10.5, range 5.5–16) than group 2 (median score of 5.5, range 2–9; $p=0.005$). In addition, mean albumin concentration was lower in group 1 than in group 2 dogs (group 1: 22.3 SD 8.1 g/l; group 2: 30.7 SD 3.8 g/l).

In conclusion, TLR2 and TLR4 expression appears to be up-regulated in dogs with severe IBD when compared to dogs with milder disease and controls. The marked elevation in TLR2 and TLR4 expression in a group of severely diseased dogs suggests that an abnormal immune response to TLR ligands may provide an excess of inflammatory mediators during the active phase of canine IBD.

ABSTRACT #27

EFFECT OF THYROXINE SUPPLEMENTATION ON GLOMERULAR FILTRATION RATE IN HYPOTHYROID DOGS.

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Glomerular filtration rate (GFR) is decreased in human hypothyroid patients, but information about kidney function in canine hypothyroidism is lacking. The objective of this study was to assess GFR in hypothyroid dogs, prior to substitutional therapy and after reestablishment of a euthyroid state.

Hypothyroid dogs (n=14) without gross abnormalities on renal ultrasonography and urinalysis were included. Blood pressure measurement and exogenous serum creatinine clearance (ECC) test were performed before treatment (t0, n=14), one month (t1, n=14) and 6 months (t6, n=11) after supplementing levothyroxine (20 µg/kg/day PO) therapy. At t1, response to therapy was monitored by measurement of serum total thyroxine and tyrotropin. If thyroid treatment needed to be adjusted, it was reassessed after one month.

Statistical analysis was performed using a general linear model, results were expressed as mean±SD.

Age at t0 was 6.25±1.4 years, body weight decreased ($P<0.01$) from 35±18 kg at t0 to 27±14 kg at t6. All dogs remained normotensive throughout the study. Basal serum creatinine also decreased ($P<0.05$) from 121±37 to 98±20 and 104±28 µmol/L at t0, t1 and t6, respectively. ECC conversely increased ($P<0.01$), the corresponding values were 1.6±0.4, 2.1±0.4 and 2.0±0.4 mL/min/kg, respectively.

Decreased GFR was observed in hypothyroid dogs. However re-establishment of a euthyroid state increased GFR significantly.

ABSTRACT #28

GLUTAMIC ACID DECARBOXYLASE-65 (GAD65) AUTOANTIBODY STATUS AND MHC CLASS II POLYMORPHISM IN 100 DIABETIC DOGS.

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Previous work has demonstrated an association between certain Major Histocompatibility Complex (MHC) Class II alleles and susceptibility to canine diabetes mellitus (DM). Additionally, a pilot study has provided preliminary evidence that some diabetic dogs have circulating autoantibodies to glutamic acid decarboxylase 65 (GAD65), a pancreatic beta cell protein and important autoantigen in human DM. Such findings imply that autoimmunity might play a role in canine DM; however the correlation between canine MHC haplotype and autoantibody status has not been investigated in dogs. The current study was designed to determine the prevalence of GAD65 autoantibodies in a large cohort of diabetic dogs for whom no other underlying cause of DM was obvious. In addition, the study aimed to test the hypothesis that dogs with GAD65 autoantibodies would share one or more of the canine MHC Class II haplotypes associated with increased DM risk.

The previously described canine GAD65 autoantibody assay was refined by cloning the canine GAD65 gene into the pVAX expression vector, to improve *in-vitro* transcription / translation of ^{35}S methionine radio-labelled GAD65 from plasmid DNA. Sera from canine diabetic patients (n=100) and normoglycaemic control patients (n=45) were screened for GAD65 autoantibodies by radioimmuno-precipitation. Diabetic patients who were under 6 months old, female entire, or had a known history of pancreatitis were excluded, since autoimmune pancreatic destruction was thought to be an unlikely underlying cause of DM in these dogs. DNA was also isolated from the canine diabetic blood samples and genotyped at the Dog Leukocyte Antigen (DLA)-DRB, -DQB and -DQA loci using a sequence-based approach. Serum samples were considered positive for GAD65 autoantibodies if their reactivity was greater than the mean + 2 standard deviations of the controls tested.