VETERINARSKI ARHIV 86 (1), 77-94, 2016

Clinical findings, laboratory data and outcome in dogs with spontaneous hyperadrenocorticism in Croatia

Ivana Kiš[1]*, Mirna Brkljačić[1], Marin Torti[1], Iva Mayer[1], Iva Šmit[1], Jelena Gotić[1], Dražen Vnuk[2], Valentina Gusak[2], Veljko Turković[3], and Vesna Matijatko[1]

[1]*Clinic for Internal Diseases, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

[2]*Clinic for Surgery, Orthopaedics and Ophtalmology, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

[3]*Veterinary Small Animal Clinic Hofheim, Hofheim, Germany*

KIŠ, I., M. BRKLJAČIĆ, M. TORTI, I. MAYER, I. ŠMIT, J. GOTIĆ, D. VNUK, V. GUSAK, V. TURKOVIĆ, V. MATIJATKO: Clinical findings, laboratory data and outcome in dogs with spontaneous hyperadrenocorticism in Croatia. Vet. arhiv 86, 77-94, 2016.

ABSTRACT

Hyperadrenocorticism (HAC) is one of the most common endocrine disorders in dogs, and establishing a diagnosis requires extensive diagnostics. In this study, 54 dogs with spontaneous hyperadrenocorticism were included, and were divided in two groups: the treated group which consisted of 35 dogs (trilostane therapy 32, adrenalectomy 3) and the non-treated group, which consisted of 19 dogs. According to the Kaplan-Meyer survival curve, the mean survival time of the whole population was 29.6 months, where the untreated patients' mean survival time was 12.7 months, and in patients with therapeutic intervention it was 34.7 months. In the multivariable Cox proportional hazards model, no single clinical or laboratory parameter was found to significantly influence the outcome. The significance of this research is in its contribution to understanding the natural course of spontaneous canine hyperadrenocorticism.

Key words: canine adrenal gland, diagnostic procedures, outcome

Introduction

Hyperadrenocorticism (HAC), or Cushing's syndrome, is one of the most common endocrine disorders in dogs, and is characterized by chronically increased circulating cortisol concentration, which causes both physical and biochemical changes (KOOISTRA

*Corresponding author:

Assist. Prof. Ivana Kiš, PhD, DVM, Clinic for Internal Diseases, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia, Phone +385 1 2390 350; E-mail: ivana.kis@vef.hr.

ISSN 0372-5480
Printed in Croatia

and GALAC, 2012). Clinical presentation consists of an almost pathognomonic constellation of clinical signs (polyuria/polydipsia, polyphagia, muscle wasting, "potbelly", thinning of the skin and bilaterally symmetrical alopecia), but the more subtle cases of Cushing's syndrome can be difficult to diagnose, and the interpretation of dynamic adrenal function tests is complicated when other diseases are present (GILOR and GRAVES, 2011). HAC has an insidious onset and is slowly progressive over many months or even years. Most owners consider the early signs as part of the normal aging process of their dog. In a few cases, clinical signs may be intermittent, with periods of remission and relapse, whereas in others there may be an apparent rapid onset and progression of clinical signs. Thus, making a diagnosis requires considerable clinical insight (KOOISTRA and GALAC, 2012; PETERSON, 2007).

The syndrome can be caused by oversecretion of the adrenocorticotropic hormone (ACTH) from a pituitary abnormality (pituitary-dependent HAC or PDH), the result of a primary adrenocortical tumour (adrenal-dependent HAC or ADH) or iatrogenic administration of glucocorticoids (HERRTAGE and RAMSEY, 2012). A presumptive diagnosis of HAC can be made from clinical signs, physical examination, routine laboratory tests, and diagnostic imaging findings, but the diagnosis must be confirmed by hormonal assay (BEHREND et al., 2013). Regardless of which test is used, a high degree of clinical suspicion is mandatory to avoid false-positive test results (BEHREND et al., 2013; KOOISTRA and GALAC, 2012; PETERSON, 2007).

Ultrasonography has been determined to be a useful primary screening modality to identify abnormal adrenal glands (CHOI et al., 2011; GROOTERS et al., 1994; GROOTERS et al., 1996) and testing for HAC is recommended after identification of bilateral adrenomegaly or unexpected identification of an adrenal mass on imaging performed for another problem (BEHREND et al., 2013).

Treatment of HAC depends on whether it is PDH or ADH. At the level of the pituitary gland PDH can be treated surgically with transsphenoidal hypophysectomy (VAN RIJN et al., 2015) in which, when the surgeon has acquired the necessary experience, the results compare favourably with those of chemotherapy using mitotane. This procedure can only be performed in specialized institutions. To date several attempts have been made to medically reduce pituitary hypersecretion of ACTH, and these investigations have reported clinical improvement but have not been followed by broader use (GALAC et al., 2010). In everyday practice, medical treatment is aimed at the adrenal level to reduce the glucocorticoid excess, either medically or surgically. The two most commonly used medications are mitotane and trilostane (HELM et al., 2011). Treatment with trilostane has become the medical treatment of choice (PEREZ ALENZA et al., 2006; GALAC et al., 2009). For ADH in cases of resectable adrenal tumours, adrenalectomy is the recommended

treatment, but in cases of non-resectable tumours, medical treatment with trilostane is the treatment of choice (ARENAS et al., 2014).

Since HAC presents a significant endocrinopathy in dogs, and treatment regimes have changed over time, as well as prognosis of the disease, the aim of the present study was to determine the clinical and laboratory findings present in spontaneous canine HAC, compare them to outcome, and evaluate epidemiologic, clinical and laboratory findings that could be associated with survival time. In addition, we hypothesized that treated dogs (with trilostane or adrenalectomy) would have significantly longer survival time compared to dogs for which no treatment was instituted. Furthermore, to the authors' knowledge there were no previously published studies dealing with hyperadrenocorticism in Croatia. Therefore we wanted to compare results obtained from our population with the results of other studies.

Materials and methods

Clinical cases. All the dogs diagnosed with spontaneous HAC at the Clinic for Internal Diseases of the Veterinary Faculty in Zagreb, between August 2009 and August 2014, were included in this study. Before analysing the data, we assigned the patients to two groups: the non- treatment and the treatment group. The decision not to treat the dogs was made by their owners predominantly due to financial reasons. The following data were analysed: epidemiological [breed, sex, weight, size of the dog according to body weight (<10 kg, and >10 kg), age at diagnosis (< 10 years, and >10 years)], selected clinical signs (polyuria/polydipsia, polyphagia, abdominal distention, liver enlargement, muscle weakness, lethargy, panting, billateraly symmetrical alopecia, calcinosis cutis, gonadal atrophy, neurological signs and hypertension), concomitant diseases (diabetes mellitus, urinary tract infection), results of abdominal ultrasound examination, and from routine blood analysis selected haematological (haematocrit, thrombocytes and white blood cell count) and biochemical parameters: alkaline phosphatase, alanine aminotransferase, aspartat aminotransferase, glucose, urea, creatinine, cholesterol and triglycerides). Bacteriological examination of the urine was performed at the time of diagnosis. In patients that were hospitalised, cystocentesis was performed, and in patients that were treated as outpatients, a spontaneous voided sample was collected. For voided samples quantitative as well as qualitative bacteriological examination was performed. For all cases concurrent diseases, at presentation or during study as detected by clinical examination, complete blood count, biochemistry profiles, and imaging modalities were recorded. Additionally, blood pressure measurements were performed at the time of diagnosis using the Doppler oscillometric system. Hypertension was defined as a systolic blood pressure >160 mm Hg (BROWN et al., 2007).

Diagnostic procedures. The presumptive diagnosis of HAC was made on the basis of clinical history, physical examination, ultrasonography findings, and the results of haematology, serum biochemistry and urinalysis findings. The diagnosis of HAC was confirmed, either by a combination of two adrenal function tests [in which case one had to be low-dose dexamethasone suppression test (LDDST)] or at least one positive endocrine test (ACTH stimulation test or LDDST) combined with the positive ultrasonography findings. Results of the ACTH stimulation test were considered positive when post-ACTH cortisol concentration was > 600 nmol/l, and LDDST results were considered positive if at 8 hours following suppression there was no suppression to below 40 nmol/l (HERRTAGE, 2004). Individually performed diagnostics are showed in Table 1.

Table 1. Combinations of diagnostic methods applied in establishing a diagnosis of spontaneous HAC in researched population (n = 54).

Diagnostic test	Number of patients
US + LDDST	8
US + ACTH stimulation test	34
US + UCCR	1
LDDST + ACTH stimulation test	11
Total	54

Endocrinologic tests were considered to be positive according to values proposed by HERRTAGE (2004). US - ultrasound examination, LDDST - low dose dexamethasone supression test, ACTH stimulation test - adrenocorticotropic hormone stimulation test, UCCR - urine cortisol creatinine ratio.

Adrenal function tests. For the ACTH stimulation test, plasma cortisol concentrations were measured before and 1 hour after i/v injection of 250 μg tetracosactrin (Synacthen, Novartis). LDDST consisted of measurements of cortisol concentrations before and 4 and 8 hours after administration of 0.01mg/kg dexamethasone (Dexamethasone, Alfasan International). For UCCR two consecutive urine samples were collected in which creatinine and cortisol concentrations were measured (Synlab, Germany, Augsburg). Cortisol was measured by external commercial laboratories by radioimmunoassay or immunoradiometric assay validated for use in dogs, with appropriate quality controls.

Diagnostic imaging. Forty-two patients were examined by ultrasonography (MyLab™ 40, Esaote) with a 5-8 MHz curved array transducer, to determine the size of the adrenal glands. For the diagnosis of HAC both glands had to be of normal shape, echogenicity and of symmetrical size, with less than 5 mm difference in thickness between glands. Measurements were made on the caudal pole of the adrenal glands, and for dogs weighing > 10 kg the cut-off value was set at 7.4 mm (BARBERET et al., 2010; PEY et al., 2012), and for dogs weighing < 10 kg the cut-off value was 6.4 mm (CHOI et al., 2011). For diagnosis of ADH, a large solitary and abnormally shaped adrenal mass, with or without invading adjacent structures and with a difference of more than 5 mm between adrenal gland

measurement values, had to be established. The presence of thrombi and intraabdominal metastases to adjacent organs (e.g. lymph nodes, kidney and or liver) were recorded. Tumour staging was based on the findings of abdominal ultrasonography and thoracic radiography.

Trilostane therapy. Thirty-two dogs were included in the trilostane (Vetoryl, Dechra) treatment group. The initial dose was between 2 and 3 mg/kg PO q 24 h, and was adjusted according to the clinical status and the results of the ACTH stimulation test.

Dogs treated with trilostane were reviewed one week after the start of treatment, and at the 1st, 3rd, and 6th month after treatment, and then every 6-12 months thereafter. At each evaluation, history, physical examination, complete blood work and urinalysis and an ACTH stimulation test or UCCR were performed. ACTH stimulation tests were performed between 2 and 6 hours post pill in dogs in which the treatment regimen was once daily, and in those receiving therapy twice daily, at the time of administration of morning dose.

Adrenalectomy. Three dogs with ADH underwent adrenalectomy. During induction of general anaesthesia all the dogs received a single injection of cefazolin sodium (22 mg/kg i/v) as antimicrobial prophylaxis. The dogs were also treated with dexamethasone (1 mg/kg i/v) prior to anaesthesia, and continued to receive glucocorticoids parenterally until they started to eat. In all dogs anaesthesia was induced with slow i/v injections of fentanyl (2 μg/kg i/v) and propofol (4 mg/kg i/v) via an i/v catheter, followed by maintenance with isoflurane in oxygen after endotracheal intubation (MASSARI et al., 2011). Midline laparotomy was performed and the adrenal glands were removed via gentle dissection. Haemostasis was achieved via manual vessel ligation or bipolar electrocautery, or a blood vessel sealing device (Ligasure, Covidien, USA). If the tumour had invaded the kidney or a thrombus had obstructed the renal vein, concurrent nephrectomy was performed (1 dog). After surgery, as standard care all the dogs were given i/v crystalloid fluids, cefazolin (22 mg/kg i/v) and analgesic drugs (fentanyl 2-3 μg/kg/h i/v) progressively tapered and discontinued when the dogs no longer had signs of abdominal pain on gentle abdominal palpation. Administration of prednisolon (1 mg/kg PO, q24h) was started after the dog began to eat and drink, which was between 24-48 hours post operation.

Histopathology was performed on samples obtained by laparotomy and they were classified as either benign (adenoma) or malignant (carcinoma) according to vascular invasion, the presence of high mitotic rate, increased nuclear pleomorphism, a high percentage of tumour necrosis or some combination of these findings (LABELLE et al., 2005).

Statistical analyses. For the descriptive analyses of continuous data, the mean, standard deviation, and the range of values are reported. For categorical data, frequencies and percentages are given. The population characteristics of both groups were compared using the chi-square test for sex, breed, concurrent illness and clinical signs. Survival

time analysis was performed using a Kaplan-Meier survival curve, with the dogs still alive at the end of the study being censored. The log-rank test was used to compare survival curves of the 2 groups. Overall survival was defined as the time from establishing the diagnosis, or from the beginning of treatment, to the day of death. Univariate Cox proportional hazard regression analysis was performed to screen potential predictor factors for subsequent inclusion in a multivariate model. Potential predictive variables including: epidemiologic factors (age, sex, breed, breed size, reproductive status and weight), clinical signs detected at diagnosis (polyuria/polydipsia, polyphagia, abdominal distention, liver enlargement, weakness, lethargy, panting, dermatologic signs, concurrent diseases, neurologic signs and blood pressure) laboratory findings, and the presence of therapy were analysed.

To identify the independent impact of variables on the survival time of the dogs, multivariate analysis was performed. The variables tested for their impact on overall survival were those significantly correlated at the univariate level. Hazard ratios (HR) with a 95 % confidence interval (CI) were calculated. For all the statistical analyses $P \leq 0.05$ was considered significant.

Results

Fifty-four dogs were included in this study. There were 30 female dogs (12 intact and 18 spayed) and 24 male dogs (18 intact and 6 neutered). There were 22 breeds represented in this investigation and their distribution is presented in Table 2. PDH was diagnosed in 41 (75.9 %) dogs, and ADH in 13 dogs (24.1 %).

Table 2. Breeds represented in this study.

Breed	Number of patients
Cross breed	19
Labrador retriever	4
Yorkshire Terrier	3
West Highland White Terrier	3
German boxer	3
Poodle	3
Maltese Terrier	2
Samoyed	2
Italian Water Dog	2
Alaskan Malamute, American Staffordshire Terrier, Beagle, Bernese Mountain dog, Bichon Frisee, Croatian Shepherd Dog, Dalmatian, English Cocker Spaniel, German Shepherd Dog, German Short-haired Pointer, Golden Retriever, Irish Setter, Scottish Terrier	1 of each (total 13)
Total	54

The mean age at the time of diagnosis was 10 years. The mean age at admission in the group of treated dogs was 10.2 years, and for those in group of non-treated dogs was 9.8 years. Mean body weight was 21.6 kg, ranging from 2.7 to 46 kg, and for treated dogs it was 21.8 kg in a range from 5 kg to 44.4 kg, and for those in non-treated group 21.2 kg, ranging from 2.7 kg to 46 kg. Of epidemiologic factors only breed size was found to be statistically significant (log rank P = 0.034).

Table 3. Categorical clinical values of investigated population (n = 54), treatment group (n = 35), and non-treatment group (n = 19) and results of Log rank analysis

Clinical signs	Population	Treatment group	Non-treatment group	Log rank P value
Polyuria/polydipsia	44 (81 %)	31 (89 %)	13 (68 %)	0.070
Polyphagia	23 (43 %)	17 (49 %)	6 (32 %)	0.619
Abdominal distention	33 (61 %)	22 (63 %)	11 (58 %)	0.571
Liver enlargement	36 (67 %)	26 (74 %)	10 (53 %)	0.754
Muscle weakness	28 (52 %)	18 (51 %)	10 (53 %)	0.112
Lethargy	15 (28 %)	10 (29 %)	5 (26 %)	0.373
Panting	38 (70 %)	24 (69 %)	14 (74 %)	0.491
Billateraly symmetrical alopecia	26 (48 %)	18 (51 %)	8 (42 %)	0.873
Calcinosis cutis	4 (7 %)	2 (6 %)	2 (11 %)	0.167
Gonadal atrophy	18/30 (60 %)	13/18 (72 %)	5/12 (42 %)	0.749
Neurological signs	5 (9 %)	4 (11 %)	1 (5 %)	**0.039**
Hypertension	42 (76 %)	28 (80 %)	14 (74 %)	0.113
Urinary tract infections	28 (52 %)	17 (49 %)	11 (58 %)	0.435
Diabetes mellitus	7 (13 %)	6 (17 %)	1 (5 %)	0.654
Concurrent diseases:	17 (31 %)	8 (23 %)	9 (47 %)	0.084
Renal insufficiency	4 (7 %)	1 (3 %)	3 (16 %)	
Respiratory infection	1 (2 %)	0	1 (5 %)	
Mammary gland carcinoma	3 (6 %)	3 (9 %)	0	
Mitral insufficiency	6 (11 %)	3 (9 %)	3 (16 %)	
EPI	1 (2 %)	0	1 (5 %)	
HCM	1 (2 %)	1 (3 %)	0	
Liver tumor	1 (2 %)	0	1 (5 %)	

Gonadal atrophy was considered to be present in bitches with cycle irregularities and in males having soft and spongy testicles on physical examination. EPI - Exocrine pancreatic insufficiency, HCM - Hypertrophic cardiomyopathy.

The results of clinical categorical data are presented in Table 3. The most frequently encountered clinical sign was polydipsia and polyuria, followed by panting and by abdominal distension and liver enlargement. With decreasing frequency, polyphagia, weakness, lethargy and dermatological signs were observed. The only statistically significant difference was the presence of neurological signs (log rank P = 0.039).

The most commonly encountered concurrent disease was diabetes mellitus, which was present in 7 patients (13 %), of those 6 (17 %) were in the treatment, and one in the non-treatment group (5 %). All the patients with diabetes mellitus were treated with recombinant human NPH insulin twice daily and controlled with serial glucose curves, according to NELSON (2010). The presence of concurrent diseases was not found to be statistically significant (log rank P = 0.084).

Selected standard laboratory parameters are presented in Table 4. Most of the patients had some combination of changed laboratory parameters that often accompany HAC, such as lymphopenia, eozinopenia, thrombocytosis and increased values of alkaline phosphatase, glucose, cholesterol and triglycerides. However, only the presence of thrombocytosis was found to be statistically significant (log rank P = 0.014).

There were 42 dogs (76 %) with hypertension in this study and their mean systolic arterial pressure (mSAP) was 219.19 ± 29.55 mm Hg, range 164-270 mm Hg. Of those 28 (80 %) were in the treatment group (range: 164 - 270 mm Hg, mSAP 209.50 ± 31.49 mm Hg), and 14 (74 %) in the non-treatment group (range: 172 - 264 mm Hg, mSAP 223.14 ± 31.49 mm Hg). All the dogs in the treatment group and 6 dogs in the non-treatment group were treated with either enalapril or amlodipine, or with a combination of these according to CARR (2010).

An adverse reaction to the medical therapy were observed in 8 (22.8 %) patients who had one or more episodes of weakness, loss of appetite, vomiting or diarrhoea at different times throughout the study. Most patients responded well to administration of i/v fluids and discontinuation of the medication. In 3/8 patients, electrolyte disturbance was recorded and glucocorticoids were administered for a short term. No patient needed permanent discontinuation of the therapy or long-term glucocorticoid treatment, however 2 patients were euthanized after repeated episodes of adverse reactions.

Thirteen dogs presented with a unilateral adrenal mass (24.1 %), of which 6 were on the left (6/13, 46 %), and 7 (7/13, 54 %) on the right side. The maximal dorsoventral thickness ranged from 12 to 49 mm (mean 28.83 ± 13.02 mm), in all patients there was a difference of > 5 mm between the affected and the contralateral gland. Thrombi were recorded in 6/13 (46 %). Three dogs underwent adrenalectomy, and in one nephrectomy was performed. Histopathology results were available in all 3 dogs treated surgically. One dog had adrenocortical adenoma, and the remaining two had carcinomas.

Table 4. Selected standard haematological and biochemical parameters in examined population (n = 54), treatment group (n = 35), non-treatment group (n = 19) and log rank test P values

Parameters (Units)	Reference range	Population		Treatment group		Non-treatment group		Log rank
		range	mean ± SD	range	mean ± SD	range	mean ± SD	P value
Haematocrit (%)	37-55	24-67	47.30 ± 8.73	33-67	50.11 ± 7.58	24-54	42.11 ± 8.47	0.905
Leukocytes ($\times 10^9$/L)	6.0-17	5.7-38.3	14.2 ± 7.51	5.7-38.3	14.01 ± 8.51	8.6-30.1	14.54 ± 5.41	0.131
Segmented neutrophils (%)	60-77	54-96	79.02 ± 9.29	54-94	78.37 ± 9.18	61-96	80.31 ± 9.64	0.068
Band neutrophils (%)	0-3	0-23	2.07 ± 4.68	0-23	2.46 ± 5.38	0-12	1.37 ± 3.02	0.932
Lymphocytes (%)	12-33	1-43	12.52 ± 9.06	1-43	12.34 ± 8.88	2-33	12.84 ± 9.62	0.221
Eosinophils (%)	2-10	0-12	2.15 ± 2.72	0-12	2.34 ± 2.94	0-7	1.79 ± 2.30	0.101
Monocytes (%)	3-10	0-25	4.50 ± 4.68	0-25	4.89 ± 5.11	0-17	3.79 ± 3.79	0.098
Platelets ($\times 10^9$/L)	200-700	218-794	435.31 ± 135.16	218-794	442.45 ± 145.81	235-645	422.16 ± 115.55	**0.014**
Alkaline phosphatase (U/L)	-156	30-19251	1785.26 ± 3369.06	30-5705	1154.14 ± 1286.90	91-19251	2947.84 ± 5300.17	0.106
Alanine aminotransferase (U/L)	-88	25-3616	374.63 ± 610.29	25-3616	352.09 ± 649.59	27-1793	416.16 ± 544.79	0.171
Aspartat aminotransferase (U/L)	-82	14-5353	171.59 ± 723.84	22-5353	226.91 ± 897.42	14-322	69.68 ± 67.57	0.181
Glucose (mmol/L)	3.6-6.5	2.5-32.9	7.56 ± 6.55	2.5-32.9	7.81 ± 6.71	3.0-32,9	7.12 ± 6.42	0.960
Urea (mmol/L)	3.3-8.3	1.9-38.1	7.29 ± 6.67	2.6-17.6	6.78 ± 4.18	1.9-38.1	8.24 ± 9.83	0.545
Creatinine (μmol/L)	44-140	49-244	90.17 ± 39.59	50-174	87.86 ± 31.13	49-244	94.42 ± 52.49	0.796
Triglycerides (mmol/L)	0.2-1.3	0.6-12.1	2.32 ± 2.42	0.6-12.1	2.53 ± 2.64	0.6-4.0	1.94 ± 1.19	0.304
Cholesterol (mmol/L)	3.5-7.1	2.4-21.9	10.22 ± 3.70	2.4-21.3	9.70 ± 3.46	4.6-21.9	11.17 ± 4.03	0.241

For following parameters if values were increased above upper reference limit positive categorical value was assigned for log rank test: hematocrit, leukocytes, segmented neutrophils, band neutrophils, monocytes, platelets, alkaline phosphatase, alanine aminotransferase, glucose, creatinine, triglycerides and cholesterol. For following parameters if values were decreased below lower reference limit positive categorical value was assigned for log rank test: lymphocytes, eosinophils and urea.

At the date of censorship 28 dogs were still alive. The remaining 26 dogs had died or were euthanized for the following reasons: status epileptics (n = 2) and appearance of seizures or cluster seizures (n = 2), diabetic ketoacidosis (n = 1), mammary gland carcinoma (n = 2), liver tumour (n = 1), progression of mitral insufficiency (n = 2) and dilatative cardiomiopathy (n = 1), renal insufficiency (n = 1), pneumonia (n = 2) gastrointestinal infection (n = 2), urinary tract infection (n = 1), weakness and anorexia (n = 4). For five dogs the cause of death was unknown (n = 5). In only 5 patients necropsy was performed which confirmed clinical suspicions (pituitary adenoma, liver tumour, mammary gland carcinoma, pneumonia and gastrointestinal infection).

Kaplan-Meier survival estimates. The mean survival time for the investigated population was 29.6 months (SE: 2.5; 95 % CI: 24.7-34.5 months). There was a significant difference between the survival time (Fig. 1) of treated dogs (mean 34.7; SE: 4.4; 95 % CI: 26.1-43.3 months) and untreated dogs (mean 12.7; SE: 3.3; 95 % CI: 6.2-19.1 months) (P = 0,014). At the end of the study there were 14 (40.0 %) and 12 (63.1 %) dead dogs in the treated and untreated groups, respectively (P < 0.05). The 1 year survival fraction for dogs without therapy was 33 ± 12 %, whereas for dogs with therapy it was 62 ± 9 % (P = 0.027). When comparing dogs without therapy to dogs under therapy, dogs that were not treated had 2.43 times (95 % CI: 1.01-5.83) higher chance of death compared to the treated dogs.

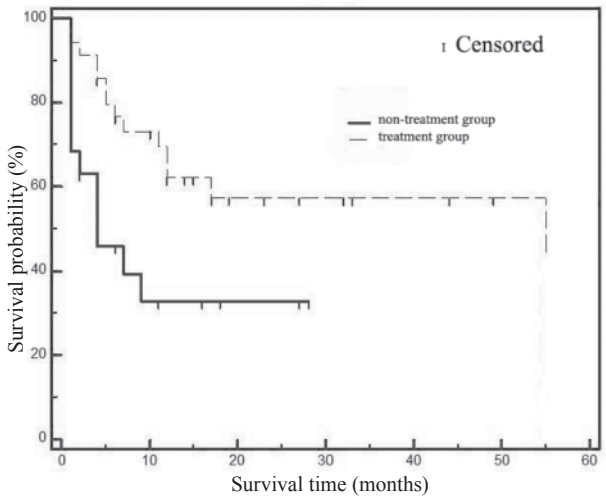

Fig. 1. Kaplan-Meier survival curve for both treated (n = 35) and non-treated (n = 19) dogs

Additionally, survival time was investigated separately for PDH and ADH, as these are two different forms of naturally occurring HAC. Of 41 dogs diagnosed with PDH (Fig 2), 30 were in the treatment group, and at the time of control there were 17 alive and 13 dead dogs. In the non-treatment PDH group there were 5 dogs alive and 6 dead. The mean survival time for dogs with PDH was 31.4 months (SE: 2.9; 95 % CI: 25.7-37.1 months). The mean survival time for treatment dogs with PDH was 33.9 months (SE: 4.7; 95 % CI: 24.7-43 months) and for the non-treatment group it was 15.5 months (SE: 4.6; 95 % CI: 6.6-24.4) but there was no statistically significant difference between the treatment and non-treatment groups (P = 0.152). Of 13 dogs diagnosed with ADH, three underwent adrenalectomy and were alive at the time of writing this manuscript. Of the remaining 10 dogs, two were in the treatment group of which 1 was alive and 1 dead, and the remaining 8 were in the non-treatment group and for them the survival curve is presented in Fig 3. The mean survival time for dogs with ADH and without treatment was 6.1 months (SE: 1.96; 95 % CI: 2.2-9.9). At the time of censorship in the non-treatment group of dogs with ADH there were 6 dogs dead and 2 alive.

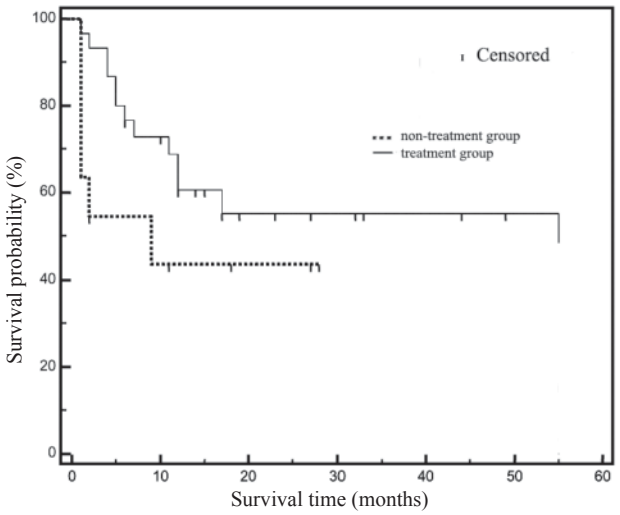

Fig. 2. Kaplan-Meier survival curve for dogs diagnosed with PDH (n = 41), both treated (n = 30) and non-treated (n = 11) dogs

Epidemiologic, clinical and laboratory variables at time of diagnosis, as well as the presence of therapy were examined for their potential influence on survival time (Table 5). In the univariable model only larger breed size negatively correlated, and the presence of

therapy positively correlated, with survival. In the multivariable model, only the presence of therapy positively correlated with outcome.

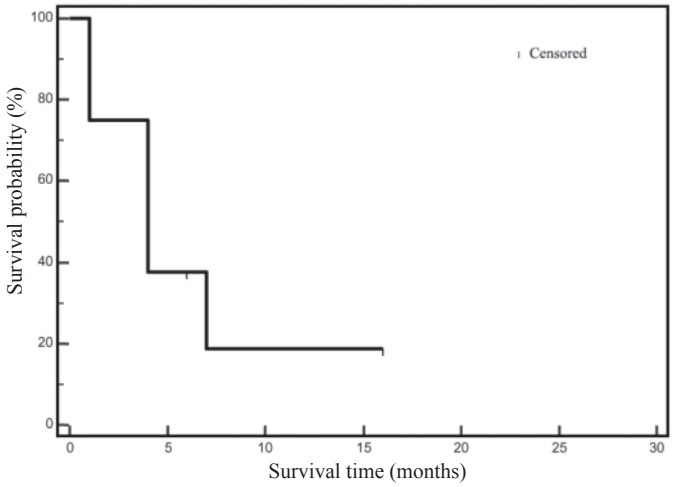

Fig. 3. Kaplan-Meier survival curve for non-treated dogs with ADH (n = 8)

Table 5. Results of Cox univariate and multivariate regression for the investigated population (n = 54)

Variable	b	SE	P	Exp (b) 95 %	CI of Exp (b)
Univariate					
Dog size (>10 kg)	0.89	0.45	0.047	2.43	1.02-5.84
Therapy	- 0.93	0.41	0.022	0.39	0.18-0.87
Multivariate					
Therapy	- 0.93	0.41	0.022	0.39	0.18-0.87

Discussion

In this study, the long-term survival time, with and without treatment, were evaluated for dogs with spontaneous HAC. Although PDH is the most common form of HAC (FELDMAN and KASS, 2012; ARENAS et al., 2014) and accounts for approximately 85 % of all cases, in this study it was diagnosed in 75.9 % (41/54) of all patients, which is compatible with the overall weight of the dogs (21.6 kg), suggesting that this population is not a classical small breed population affected by HAC, which is also in accordance with the breeds represented in this study. The reason for this difference might be that the more "classical" presenting cases of spontaneous HAC are more easily recognised by

first opinion practices, but also our investigated population may be truely representative of naturally occurring HAC in Croatia.

In our study we compared the patients in which we employed one of two recommended treatment options (trilostane for PDH and non-resectable ADH and adrenalectomy for resectable ADH), with untreated dogs. The motive for this investigation was the relatively high price of both treatment options, which was unacceptable for some owners, and the simultaneous lack of studies in which adequate diagnostic procedures were carried out, but no treatment was instituted. The results of this study suggest that there is significant difference in survival time when treatment is affordable. These findings provide information important for clinicians presented with cases of spontaneous HAC, and they may now give objective information to owners regarding the benefits of the therapy.

Of the two most commonly available medical treatment options, mitotane and trilostane, for both ADH (HELM et al., 2011) and PDH (ARENAS et al., 2014), in our study all the dogs that were medically treated were treated with trilostane, predominantly due to its availability. For dogs with PDH treated with trilostane the mean survival time in the research by NEIGER et al. (2002) was 18.3 months and in the study by BARKER et al. (2005) the median survival time was 22 months, which is shorter than in our study (33.9 months). It is important to notice that the first study had a shorter duration compared to the second and to our study, but in the first study, which lasted, three years, there was a high percentage of surviving patients (65.4 %) censored at the end of the research, and therefore this did not affect the Kaplan-Meier survival curve. In our study, due to the small number of patients with PDH, there was no statistically significant difference between treated and those that were not treated even though there was large difference in survival time. The mean survival time of patients with PDH and without therapy (15.5 months) was much longer than for patients with ADH and without therapy (6.1 months), which corresponds to the differences between pituitary and adrenal tumours (GALAC et al., 2010).

Even though therapy with trilostane is considered to be associated with less worrisome adverse effects, complications of the therapy have been reported and have included anorexia, vomiting, diarrhoea, weakness, shivering (PEREZ ALENZA et al., 2006) or unusual listlessness (FELDMAN, 2011). In our study, there were 22.8 % adverse reactions present, which is less than in the investigation by PEREZ ALENZA et al. (2006), in which 25 % similar adverse reactions were observed. FELDMAN (2011) reports that in the review of eight studies on 191 dogs treated once daily with trilostane, 22 % of patients were sufficiently ill to have the adverse effects reported, which is in agreement with our results.

In our investigated population, comorbidites were the cause of euthanasia or death in 7 patients, in which no direct correlation with HAC could be established. However,

all the dogs that succumbed to inflammatory processes, as well as the dog with diabetic ketoacidosis, were in the non-treatment group where it could be presumed that HAC had some contributory role. Furthermore, all the patients that had neurological signs present at presentation, or where they emerged during therapy, were diagnosed with PDH. For those dogs it was presumed that there had been growth of a pituitary adenoma, which was confirmed in one case where necropsy was allowed. For dogs presenting with weakness and loss of appetite (n = 4), therapy complications were suspected. Two of those were euthanized without further diagnostics, and in two, clinical findings and electrolyte abnormalities indicated therapy complications but the owners declined hospitalisation and the patients were euthanized.

There is only a limited number of studies in veterinary medicine that have tried to evaluate prognostic factors for dogs with ADH (HELM et al., 2011; ARENAS et al., 2014) and PDH (NEIGER et al., 2002; BARKER et al., 2005). In the work by NEIGER et al. (2002) no statistically significant influence on survival time was found after testing age, weight, initial dose of therapy and post-ACTH cortisol concentration. Conversely in the work by BARKER et al. (2005), age and weight at diagnosis were significantly negatively associated with survival. Age and post-adrenocorticotropic cortisol concentrations, together with weakness at presentation, were found to be statistically significant in the work by ARENAS et al. (2014), while in the study of HELM et al. (2011) the only prognostically significant factor was the presence of metastatic disease. Of potential prognostic factors in our study only dog size, the presence of neurological signs and thrombocytosis were found to be significant in log rank analysis. However, in the Cox regression model no clinical or laboratory parameters were found to significantly influence the outcome. The lack of a few statistically significant predictive factors might be explained by the endocrine nature of the disease with cortisol effects influencing all body systems, in which the clinical picture is the summation of all the effects of the circulating cortisol (ARENAS et al., 2014).

Adrenalectomy reportedly has an overall high complication rate, ranging to as high as 51 % (SCHWARTZ et al., 2008), with the most common complications including postoperative adrenal gland insufficiency, pulmonary thromboembolism, pancreatitis and acute renal failure (KYLES et al., 2003; SCHWARTZ et al., 2008). However, patients surviving to discharge achieve a good long-term outcome, with a median survival time of more than 10 months (MASSARI et al., 2011). In this study, all three dogs that underwent adrenalectomy were alive at the time of writing this paper.

It may be concluded that the findings of this study have proven that the current recommended treatment significantly prolongs the life expectation of patients with HAC. To the best of the authors' knowledge there is no study evaluating the long-term outcome of HAC patients without therapy.

One limitation of this study was the lack of control appointments for non-treatment group patients dedicated to clinical and endocrinological evaluation, which would enable us to better understand the progression of clinical signs in the natural course of the disease. For the treatment group, a wide range of ACTH-stimulation tests was used initially in the study ranging from 2 to 6 hours post pill, which could have led to decisions about therapy change in some patients when the time of maximal effect of trilostane had been missed (GALAC et al., 2009).

The other limitation is that the present research included mostly middle-aged or old patients, in which there is an increased chance of concurrent diseases. Although we excluded all patients with spontaneous HAC euthanized due to concurrent illness, it is possible that in some cases concurrent diseases influenced the outcome. Still, it is reasonable to expect that in every population of spontaneous HAC we would investigate a certain proportion of cases with concurrent diseases.

Finally, in veterinary medicine, the recommended treatment modalities prolonged the life of our patients in comparison to the natural course of the disease. Additional large clinical studies are required to better evaluate the clinical signs and possibly find new parameters that could be used as prognostic factors for naturally occurring hyperadrenocorticism.

References

ARENAS, C., C. MELIÁN, M. D. PÉREZ-ALENZA (2014): Long-term survival of dogs with adrenal-dependent hyperadrenocorticism: a comparison between mitotane and twice daily trilostane treatment. J. Vet. Intern. Med. 28, 473-480.

BARBERET, V., P. PEY, L. DUCHATEAU, A. COMBES, S. DAMINET, J. H. SAUNDERS (2010): Intra- and interobserver variability of ultrasonographic measurements of the adrenal glands in healthy Beagles. Vet. Radiol. Ultrasound 51, 656-660.

BARKER, E. N., S. CAMPBELL, A. J. TEBB, R. NEIGER, M. E. HERRTAGE, S. W. J. REID, I. K. RAMSEY (2005): A comparison of the survival times of dogs treated with mitotane or trilostane for pituitary-dependent hyperadrenocorticism. J. Vet. Intern. Med. 19, 810-815.

BEHREND, E. N., H. S. KOOISTRA, R. NELSON, C. E. REUSCH, J. C. SCOTT-MONCRIEFF (2013): Diagnosis of spontaneous canine hyperadrenocorticism: 2012 ACVIM consensus statement (small animal). J. Vet. Intern. Med. 27, 1292-1304.

BENCHEKROUN, G., P. DE FORNEL-THIBAUD, M. I. RODRIGUEZ PIÑEIRO, D. RAULT, J. BESSO, A. COHEN, J. HERNANDEZ, F. STAMBOULI, E. GOMES, F. GARNIER, D. BEGON, C. MAUREY-GUENEC, D. ROSENBERG (2010): Ultrasonography criteria for differentiating ACTH dependency from ACTH independency in 47 dogs with hyperadrenocorticism and equivocal adrenal asymmetry. J. Vet. Intern. Med. 24, 1077-1085.

BROWN, S., C. ATKINS, R. BAGLEY, A. CARR, L. COWGILL, M. DAVIDSON, B. EGNER, J. ELLIOTT, R. HENIK, M. LABATO, M. LITTMAN, D. POLZIN, L. ROSS, P. SNYDER, R.

STEPIEN (2007): ACVIM Consensus statement: Guidelines for the identification, evaluation, and management of systemic hypertension in dogs and cats. J. Vet. Intern. Med. 21, 542-558.

CARR, A. P. (2010): Treatment of hypertension. In: Textbook of veterinary internal medicine. (Ettinger, S. J., E. C. Feldman, Eds.), Saunders Elsevier, St. Louis, pp. 582-585.

CHOI, J., H. KIM, J. YOON (2011): Ultrasonographic adrenal gland measurements in clinically normal small breed dogs and comparison with pituitary-dependent hyperadrenocorticism. J. Vet. Med. Sci. 73, 985-989.

FELDMAN, E. C. (2011): Evaluation of twice-daily lower-dose trilostane treatment administered orally in dogs with naturally occuring hyperadrenocorticism. J. Am. Vet. Med. Assoc. 238, 1441-1451.

FELDMAN, E. C., P. H. KASS (2012): Trilostane dose versus body weight in the treatment of naturally occurring pituitary-dependent hyperadrenocorticism in dogs. J. Vet. Intern. Med. 26, 1078-1080.

GALAC, S., J. J. C. W. M. BUIJTELS, H. S. KOOISTRA (2009): Urinary corticoid: creatinine ratios in dogs with pituitary-dependent hypercortisolism during trilostane treatment. J. Vet. Intern. Med. 23, 1214-1219.

GALAC, S., C. E. REUSCH, H. S. KOOISTRA, A. RIJNBERK (2010): Adrenals. In: Clinical endocrinology of dogs and cats. (Rijnberk, A., H. S. Kooistra, Eds.), Schlütersche, Hannover, pp. 93-154.

GILOR, C., T. K. GRAVES (2011): Interpretation of laboratory tests for canine Cushing's syndrome. Top. Companion Anim. Med. 26, 98-108.

GROOTERS, A. M., D. S. BILLER, T. MIYABAYASHI, R. LEVEILLE (1994): Evaluation of routine abdominal ultasonography as a technique for imaging the canine adrenal glands. J. Am. Anim. Hosp. Assoc. 30, 457-462.

GROOTERS, A. M., D. S. BILLER, S. K. THEISEN, T. MIYABAYASHI (1996): Ultrasonographic characteristics of the adrenal glands in dogs with pituitary-dependent hyperadrenocorticism: comparison with normal dogs. J. Vet. Intern. Med. 10, 110-115.

HELM, J. R., G. McLAUCHLAN, L. A. BODEN, P. E. FROWDE, A. J. COLLINGS, A. J. TEBB, C. M. ELWOOD, M. E. HERRTAGE, T. D. H. PARKIN, I. K. RAMSEY (2011): A comparison of factors that influence survival in dogs with adrenal-dependent hyperadrenocorticism treated with mitotane and trilostane. J. Vet. Intern. Med. 25, 251-260.

HERRTAGE, M. E. (2004): Canine hyperadrenocorticism. In: BSAVA manual of canine and feline endocrinology. (Mooney, C. T., M. E. Peterson, Eds.), BSAVA, Quedgeley, pp. 150-171.

HERRTAGE, M. E., I. K. RAMSEY (2012): Canine hyperadrenocorticism. In: BSAVA manual of canine and feline endocrinology. (Mooney, C. T., M. E. Peterson, Eds.), BSAVA, Quedgeley, pp. 167-199.

KOOISTRA, H. S., S. GALAC (2012): Recent advances in the diagnosis of Cushing's syndrome in dogs. Top. Companion Anim. Med. 27, 21-24.

KYLES, A. E., E. C. FELDMAN, H. E. V. DE COCK, P. H. KASS, K. G. MATHEWS, E. M. HARDIE, R. W. NELSON, J. E. ILKIW, C. R. GREGORY (2003): Surgical management of

adrenal gland tumors with and without associated tumor thrombi in dogs: 40 cases (1994-2001). J. Am. Vet. Med. Assoc. 223, 654-662.

LABELLE, P., H. E. DE COCK (2005): Metastatic tumors to the adrenal glands in domestic animals. Vet. Pathol. 42, 52-58.

MASSARI, F., S. NICOLI, G. ROMANELLI, P. BURACCO, E. ZINI (2011): Adrenalectomy in dogs with adrenal gland tumors: 52 cases (2002-2008). J. Am. Vet. Med. Assoc. 239, 216-221.

NEIGER, R., I. RAMSEY, J. O'CONNOR, K. J. HURLEY, C. T. MOONEY (2002): Trilostane treatment of 78 dogs with pituitary-dependent hyperadrenocorticism. Vet. Rec. 150, 799-804.

NELSON, R. W. (2010): Canine diabetes mellitus. In: Textbook of veterinary internal medicine. (Ettinger, S. J., E. C. Feldman, Eds.), Saunders Elsevier, St. Louis, pp. 1782-1796.

PEREZ ALENZA, D., C. ARENAS, M. L. LOPEZ, C. MELIAN (2006): Long-term efficacy of trilostane administered twice daily in dogs with pituitary-dependent hyperadrenocorticism. J. Am. Anim. Hosp. Assoc. 42, 269-276.

PETERSON, M. E. (2007): Diagnosis of hyperadrenocorticism in dogs. Clin. Tech. Small Anim. Pract. 22, 2-11.

PEY, P., S. DAMINET, P. M. SMETS, L. DUCHATEAU, O. TRAVETTI, J. H. SAUNDERS (2012): Effect of glucocorticoid administration on adrenal gland size and sonographic appearance in beagle dogs. Vet. Radiol. Ultrasound 53, 204-209.

SCHWARTZ, P., J. R. KOVAK, A. KOPROWSKI, L. L. LUDWIG, S. MONETTE, P. J. BERGMAN (2008): Evaluation of prognostic factors in the surgical treatment of adrenal gland tumors in dogs: 41 cases (1999-2005). J. Am. Vet. Med. Assoc. 232, 77-84.

VAN RIJN, S. J., J. M. HANSON, D. ZIERIKZEE, H. S. KOOISTRA, L. C. PENNING, M. A. TRYFONIDOU, B. P. MEIJ (2015): The prognostic value of perioperative profiles of ACTH and cortisol for recurrence after transsphenoidal hypophysectomy in dogs with corticotroph adenomas. J. Vet. Intern. Med. 29, 869-876.

Received: 24 February 2015
Accepted: 25 August 2015

KIŠ, I., M. BRKLJAČIĆ, M. TORTI, I. MAYER, I. ŠMIT, J. GOTIĆ, D. VNUK, V. GUSAK, V. TURKOVIĆ, V. MATIJATKO: Klinički i laboratorijski nalazi te ishod liječenja pasa sa spontanim hiperadrenokorticizmom u Hrvatskoj. Vet. arhiv 86, 77-94, 2016.

SAŽETAK

Hiperadrenokorticizam jedna je od najčešćih endokrinoloških bolesti koja zahtijeva opsežnu dijagnostiku za postavljanje konačne dijagnoze. U ovo su istraživanje bila uključena 54 psa različitih pasmina koji su bili podijeljeni u dvije skupine: liječeni pacijenti (trilostan 32 i adrenalektomija 3) u kojoj se nalazilo 35 pasa i skupinu bez terapije, u kojoj se nalazilo 19 pasa. Prosječno vrijeme preživljavanja prema Kaplan-Meyer krivulji bilo je 29,6 mjeseci, pri čemu je pacijentima iz netretirane skupine aritmetička sredina bila 12,7 mjeseci, a u pacijenata u kojih se terapijski intervenirало 34,7 mjeseci. Multivarijantnom Cox regresijskom analizom nije ustanovljen niti jedan klinički ili laboratorijski pokazatelj koji bi značajno utjecao na ishod

bolesti. Doprinos je ovog istraživanja u postizanju boljeg uvida u prirodni tijek bolesti pacijenata sa spontanim hiperadrenokorticizmom.

Ključne riječi: pas, nadbubrežna žlijezda, dijagnostički postupci, ishod