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Effects of concurrent canine Cushing's syndrome and diabetes Mellitus on insulin requirements, trilostane dose, and survival time☆

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ABSTRACT

Trilostane and insulin requirements and survival time of dogs with concurrent naturally-occurring Cushing's syndrome (CS) and diabetes mellitus (DM) has not been fully investigated. This retrospective study evaluated trilostane and insulin doses in dogs with concurrent CS and DM compared to dogs with only CS or DM.

Additionally, a survival analysis was performed using a Kaplan-Meier survival curve. Survival time was compared through Log-rank test. Cox proportional regression method was used to screen predictor factors of death in dogs with CS, DM or concurrent CS and DM.

A total of 95 dogs were included, 47 dogs had CS, 31 dogs had DM and 17 dogs had concurrent CS and DM. After long-term follow-up, dogs with concurrent CS and DM required higher final median doses of insulin than dogs with DM [0.90 (0.73–1.1) vs 0.67 (0.55–0.73) u/kg/12 h; $P = 0{,}002$]. Conversely, the median trilostane requirements in dogs with concurrent CS and DM did not differ from the median trilostane requirements of dogs with CS [1.52 (0.76–2.80) vs 1.64 (1.19–4.95) mg/kg/day; $P = 0.283$]. No statistical difference was found for the median survival time between dogs with CS and dogs with concurrent CS and DM (1245 vs 892 days; $p = 0.152$). Although, median survival time of dogs with DM was not reached, it was longer than median survival time of dogs with CS and DM (892 days; $P = 0.002$). In conclusion, diabetic dogs with concurrent CS need higher insulin doses and have a shorter survival time compared to diabetic dogs without CS.

1. Introduction

Naturally-occurring Cushing's syndrome (CS) and diabetes mellitus (DM) are well-documented disorders in dogs that can occur independently or concurrently. High circulating concentration of glucocorticoids can increase hepatic gluconeogenesis and can cause impaired insulin action. (Gilor et al., 2016; Barbot et al., 2018). Subsequently, CS is known as one of the causes of DM in dogs (Hess, 2010; Nelson, 2015; Miceli et al., 2017) and other studies has shown that around 8–16% of dogs with CS have overt DM (Gomes Pöppl et al., 2016; Miceli et al., 2017). Dogs with diabetes secondary to CS may show a poor response to insulin treatment and they may become insulin resistant (>1.0 u/kg); furthermore, clinical signs (polyuria/polydipsia and polyphagia) could persist despite insulin treatment (Hess, 2010; Nelson, 2015).

CS and DM have similar clinical (polyuria, polydipsia and polyphagia), and laboratory presentations (i.e. increased alanine aminotransferase, alkaline phosphatase or cholesterol), and therefore the diagnosis of both diseases can be challenging when they occur concurrently, being one of them overlooked in some cases (Peterson et al., 1981; Miceli et al., 2017). In one study, among dogs with concurrent CS and DM, DM was diagnosed in 21.8% of cases, CS was diagnosed before in 40.7% and the diagnosis of both diseases were confirmed concurrently in 37.5% (Miceli et al., 2017).

The management of these concurrent disorders has not been fully investigated in dogs, and questions remain as to whether these dogs may require higher dose of insulin or trilostane. Similarly, limited information about survival time of dogs with concurrent CS and DM is available. One report showed that dogs with CS treated with cabergoline and

Abbreviations: ACTH, Adrenocorticotropic hormone; ALKP, Alkaline phosphatase; ALT, Alanine aminotransferase; CS, Cushing's syndrome; DM, Diabetes Mellitus; DVTDR, Dorsoventral thickness difference ratio; UCCR, Urinary corticoid creatinine ratio.

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retinoic acid or adrenalectomy had a longer median survival time compared to dogs with CS and concurrent DM equally treated for CS and also treated with porcine lente or detemir insulin (28 vs 14 months; $p < 0.001$) (Miceli et al., 2017). In addition, median survival time in diabetic dogs has been reported to be approximately 32 months, and diabetic dogs with CS had a shorter median survival time (21.5 months) compared to diabetic dogs without Cushing's syndrome (33.1 months) (Tardo et al., 2019). Dogs with concomitant CS and DM are more difficult to regulate than dogs with only one of these conditions. Additional knowledge of how coexisting CS and DM affects trilostane and insulin requirements may be helpful for clinicians to regulate both diseases when they present concurrently.

The aim of this study was to investigate the effect of concurrent CS and DM in the management and survival time of dogs compared to dogs that present only CS or DM.

2. Material and methods

Medical records of dogs with CS, dogs with DM and dogs with concurrent CS and DM that attended the Veterinary Teaching Hospital of the University of Las Palmas de Gran Canaria and the Clínicia Veterinaria Atlántico (Las Palmas de Gran Canaria, Spain) were reviewed.

The presumptive diagnosis of CS was based on clinical history, physical examination, ultrasonographic findings, and laboratory results. The diagnosis of CS was confirmed by hormonal functional tests: adrenocorticotropic hormone (ACTH) (Nuvacthén Depot®, Sigma Tau, Spain) stimulation test, or low-dose dexamethasone suppression test (LDDST). Results were considered consistent with CS if cortisol concentration was >18.0 μg/dL one hour after ACTH administration, or if cortisol was ≥1.4 μg/dL 8 h after the administration of a low dose of dexamethasone (Arenas et al., 2014; Sieber-Ruckstuhl et al., 2015). When dogs were considered highly suspicious of CS but ACTH stimulation test and LDDST showed test results within the normal limits, a persistently elevated urinary corticoid creatinine ratio (UCCR) was used to confirm the diagnosis (Behrend et al., 2013). Dogs with CS were included independently of type of CS, although recorded ultrasonographic images of the adrenal glands were used to do an ultrasonographic classification as follows: 1) Dogs were considered consistent with pituitary dependent CS if they showed symmetrical adrenomegaly without signs of malignancy. Adrenomegaly was defined as maximum dorsoventral thickness of the adrenal gland (measured in the sagittal plane) above the upper limit of the reference intervals proposed by (Melián et al., 2021). Adrenomegaly was considered symmetrical if the dorsoventral thickness difference ratio (DVTDR) was <20%; DVTDR was defined as the difference between the maximal dorsoventral thickness of the larger gland (LDV) and the maximum dorsoventral thickness of the smaller gland (SDV) with respect to the mean: DVTDR = [2(LDV-SDV)/(LDV + SDV)] x 100 as described by Benchekroun et al., 2010. In addition, we included in this group one dog that presented normal adrenal thickness (within the upper half of the reference interval) with DVTDR <20%. Although this dog did not present adrenomegaly, CS was confirmed by typical clinical signs (polyuria, polydipsia, polyphagia and calcinosis cutis) and 1-h post-ACTH cortisol concentration of 30 μg/dL. This dog showed a good clinical response and resolution of clinical signs within six months after starting trilostane treatment. Since neither an adrenal tumor nor equivocal adrenal asymmetry (EAA) were observed at ultrasound examination, this dog was considered more likely as pituitary dependent CS. 2) Dogs were considered consistent with adrenal dependent CS if they showed unilateral adrenomegaly and atrophy of the contralateral adrenal gland, as well as dogs that had unilateral or bilateral adrenomegaly with ultrasound features consistent with malignancy (ill-defined margins, loss of normal shape, invasion of adjacent structures, and/or suspected metastasis). Adrenal atrophy was considered when the maximum dorsoventral thickness of the smaller adrenal gland was below the lower limit of the reference intervals proposed by Melián et al., 2021. 3) Dogs were not classified as pituitary or adrenal dependent CS if they did not have ultrasonographic images of the adrenal glands recorded from diagnosis, or if they presented equivocal adrenal asymmetry (EAA). Dogs with EAA showed unilateral or bilateral adrenomegaly with a DVTDR ≥20% (Benchekroun et al., 2010), no signs of adrenal gland atrophy in the smaller gland and no features consistent with malignancy.

The diagnosis of DM was based on blood glucose concentration ≥ 200 mg/dL together with typical clinical signs of DM (without other possible cause) (Niessen et al., 2022).

Dogs were excluded if they did not have definitive diagnosis of CS or DM or date of diagnosis were not available on clinical records. Other exclusion criteria were follow-up shorter than 30 days, a severe concurrent disease (e.g. extra-adrenal or extra-pituitary neoplasia, acute or refractory heart failure) at the time of the diagnosis of CS or DM, refusal of treatment, or transient DM.

Signalment and biochemical parameters such as alanine aminotransferase (ALT), alkaline phosphatase (ALKP) and glucose were recorded. Additionally, total initial and final daily dose (mg/kg) (at last revaluation) of trilostane (Vetoryl®, Dechra, Barcelona, Spain) and frequency of administration were recorded whenever possible in dogs with CS and in dogs with concurrent CS and DM. In dogs with DM and in dogs with concurrent CS and DM, initial and final porcine-derived lente-type insulin (Caninsulin®, MSD, Salamanca, Spain) dose (units per kilogram per injection) and frequency of administration were recorded. Dogs that received a different type of insulin were excluded for the statistical analysis of insulin dose.

2.1. Statistical analyses

Distribution of the quantitative variables was assessed through Shapiro-Wilk Test and Q-Q plots. Data were non-normally distributed and were expressed as median and interquartile range (25-75th percentile). Percentages were compared using the chi-square ($\chi 2$) test and medians were compared using the pairwise Mann-Whitney's *U* test for independent samples or using Wilcoxon's signed rank test for paired samples.

Survival analysis was performed using a Kaplan-Meier survival curve. Log-rank test was used to compare the survival curves between two groups. Survival time was defined as the time from diagnosis to death. In dogs with concurrent CS and DM, if both diseases were not confirmed simultaneously, the time of diagnosis was considered at the time the first diagnosis (either CS or DM) was confirmed. The diagnosis of both diseases was considered at the same time when they were diagnosed simultaneously or with a difference of <30 days between both diagnoses. Dogs alive or dogs lost to follow-up were censored. Univariate Cox proportional hazard regression analysis was performed to search predictor factors of death in dogs with CS, DM or concurrent disorders.

Statistical significance was set at $P < 0.05$. Data were analyzed with SPSS Statistics Version 27.0 (IBM, Madrid, Spain).

3. Results

A total of 136 medical records of dogs that presented CS, DM or concurrent CS and DM were reviewed. Among them, 41 dogs were excluded: 27 with a follow-up shorter than 30 days, nine dogs without date of diagnosis recorded, three dogs were excluded due to concurrent severe diseases at diagnosis of DM or CS (one dog presenting a mast cell tumor; one dog with a thyroid carcinoma; and other dog with respiratory problems that presented a mediastinal mass, although definitive diagnosis was not determined). In addition, one more dog was excluded due to transient DM, and other dog because treatment was refused. Therefore, a total of 95 dogs were included (62 females of which 54.8% were neutered, 33 males of which 27.3% were neutered). In total, 47 dogs had CS (32 females, 15 males), 31 dogs had DM (20 females, 11 males) and 17 dogs had concurrent CS and DM (10 females, 7 males).

The ultrasonographic classification that considered different type of CS has been described in Table 1. Data of female neutering status among groups of dogs are given in Table 2. No association was found between female neutering status and any group of dogs (CS, DM or concurrent CS and DM) neither at the start ($p = 0.561$) nor at the end of the study ($p = 0.111$). Breeds were represented as follows: mixed dogs (33.7%), Yorkshire Terriers (25.3%), Chihuahua (6.3%), French Bulldog (5.2%) and many other different breeds (29.5%) among which each breed accounted for <5%. Age, weight and biochemical profile at diagnosis and at last clinical evaluation are given in Table 3. Recorded concurrent disease at diagnosis of CS or DM were: confirmed or suspected myxomatous mitral valve disease (MMVD) stage C cardiac disease (two dogs with CS), idiopathic epilepsy (one dog with concurrent CS and DM), mastitis (one dog with DM), chronic cystitis (one dog with DM), diet-responsive inflammatory bowel disease (one dog with DM).

Among dogs with concurrent CS and DM ($n = 17$), CS was diagnosed before DM in six (35.3%) dogs, DM was diagnosed before CS in eight (47.1%) dogs, and both disorders were diagnosed at the same time in three (17.6%) dogs. The median time between the first and the second diagnosis was 194 (90–754) days for dogs with CS as first diagnosis, and 150 (60–274) days for dogs with DM as first diagnosis.

Initial total daily dose of trilostane treatment was recorded in 43 out of 47 dogs with CS, 35 of them received trilosane at 12-h interval and eight dogs at 24-h interval. Among the 35 dogs that started receiving trilostane every 12 h, the frequency of the final daily dose remained at every 12 h in 25 dogs, whereas the frequency of the dose was changed in nine dogs (in three was totally discontinued, in other three it was changed to every 8 h, in two it was changed to every 24 h, and in other dog the final trilostane dose was administered every 72 h). Additionally, one of the 35 dogs did not have final trilostane dose recorded. Among the eight dogs initially receiving trilostane every 24 h, the final frequency was changed to every 12 h in three dogs, was permanently discontinued in two dogs, remained at every 24 h in two other dogs, and the final frequency and dose was not recorded in one dog. Additionally, in two more additional dogs, in which initial dose and frequency of trilostane were not recorded, the final trilostane doses were recorded and they were administered every 24 h.

Therefore, in total 43 dogs had a final trilostane dose recorded, among them, 28 (65.1%) dogs were receiving trilostane at 12-h interval, six (14.0%) dogs at 24-h interval, five (11.6%) dogs did not longer receive trilostane, three (7.0%) dogs received final trilostane dose every 8 h, and one (2.3%) dog every 72 h.

Pre-treatment median basal and post-ACTH cortisol concentrations in dogs with CS were higher than post-trilostane median basal and post-ACTH cortisol concentrations at last visit recorded [pre-treatment median basal cortisol: 7.0 μg/dL (3.6–10.0) vs post-trilostane median basal cortisol: 2.9 μg/dL (2.1–5.2); $p < 0.001$ and pre-treatment post-ACTH median cortisol: 27.3 μg/dL (21.8–30.0) vs post-trilostane post-ACTH median cortisol: 8.2 μg/dL (5.4–12.2); $p < 0.001$)].

Table 1
Distribution of dogs according to adrenal gland ultrasonographic findings.

		PDH	ADH	Unclassified*	Total
Dogs	CS	26	3	18	47
		55.3%	6.4%	38.3%	
	CS + DM	9	1	7	17
		53%	6%	41%	
Total		35	4	25	64
		54.7%	6.3%	38.0%	

Abbreviations: DM, diabetes mellitus; CS, naturally-occurring Cushing's syndrome; CS + DM; concurrent naturally-occurring Cushing's syndrome and diabetes mellitus; PDH,pituitary dependent hypercortisolism; ADH, adrenal dependent hypercortisolism.

* Among unclassified dogs, three dogs with concurrent CS and DM did not have adrenal gland measurements recorded at diagnosis. The other 22 dogs presented equivocal adrenal asymmetry.

Table 2
Neutering status of female dogs with Cushing's syndrome, diabetes mellitus, and concurrent Cushing's syndrome and diabetes mellitus. Data are expressed as number of dogs and percentages.

		Number of females dogs	
		Entire	Neutered
At diagnosis	CS	13	19
	$N = 32$	40.6%	59.4%
	DM	11	9
	$N = 20$	55%	45%
	CS + DM	4	6
	$N = 10$	40%	60%
	Total	28	34
	$N = 62$	45.2%	54.8%
		Entire	Neutered
End of the study	CS	13	19
	N = 32	40.6%	59.4%
	DM	3	17
	N = 20	15%	85%
	CS + DM	2	8
	N = 10	20%	80%
	Total	18	44
	N = 62	29.0%	71.0%

Abbreviations: DM, diabetes mellitus; CS, naturally-occurring Cushing's syndrome; CS + DM, concurrent naturally-occurring Cushing's syndrome and diabetes mellitus.

Among dogs with concurrent CS and DM, the initial and final total daily doses of trilostane were recorded in 15 and 16 dogs, respectively. In this group, trilostane was started at 12-h interval. The final frequency of trilostane was every 24 h in two (12.5%) dogs (in one the dose was changed from twice daily to once daily and in the other dog the initial trilostane dose and frequency were not recorded). The remaining 14 (87.5%) dogs received a final trilostane frequency of every 12 h. The median final total daily dose of trilostane did not differ between dogs with CS and dogs with concurrent CS and DM [1.52 (0.76–2.80) vs 1.64 (1.19–4.95) mg/kg; $P = 0.283$] (Fig. 1). Another comparison was made only for dogs receiving a final trilostane dose at 12 h-interval, and no statistical difference was found for the median final trilostane dose between dogs with CS and dogs with concurrent CS and DM [1.68 (1.29–3.00) vs 1.57 (1.14–4.19) mg/kg; $P = 0.644$)].

The initial lente insulin (Caninsulin®, MSD, Salamanca, Spain) dose was recorded in 17 dogs with DM and 11 dogs with concurrent CS and DM; meanwhile final lente insulin (Caninsulin®, MSD, Salamanca, Spain) was recorded in 26 dogs with DM and 14 dogs with concurrent CS and DM. The median insulin dose per injection at the start of the treatment was 0.31 (0.26–0.44) u/kg in dogs with DM and 0.46 (0.30–0.54) u/kg in dogs with concurrent DM and CS. The initial insulin dose was not statistically different between groups ($p = 0.120$), whereas dogs with concurrent CS and DM required a higher final median dose of insulin per injection compared to dogs with DM [0.90 (0.73–1.1) vs 0.67 (0.55–0.73) u/kg; $P = 0.002$] (Fig. 2). Among dogs with concurrent CS and DM, insulin requirements were compared between dogs with DM as first diagnosis recorded and dogs with CS as first diagnosis recorded. Final median insulin dose in dogs with DM as first diagnosis ($n = 7$) was higher than final median insulin dose in dogs in dogs with CS as first diagnosis ($n = 5$), [(0.74–1.10) u/kg vs 0.73 (0.54–0.87) u/kg; $P = 0.034$]. In dogs with concurrent CS and DM, among those with DM as first diagnosis recorded, median insulin requirements continued increasing during trilostane treatment [initial median insulin dose 0.52 (0.34–0.76) u/kg (n = 5) vs final median insulin dose 1.00 (0.74–1.10) u/kg (n = 7); $p = 0.043$], despite dogs showed a reduction in post-ACTH cortisol after a median follow-up of 320 (130–1015) days compared to pre-treatment median concentrations [post-trilostane post-ACTH cortisol: [7.1 (6.4–9.95) μg/dL (n = 5) vs pre-treatment post-ACTH median cortisol: 28.2 (20.9–34.2) μg/dL (n = 7); (p = 0.043)].

Table 3
Age, weight and biochemical parameters of dogs with naturally-occurring Cushing's syndrome, dogs with concurrent naturally-occurring Cushing's syndrome and diabetes mellitus and dogs with diabetes mellitus at the time of diagnosis and at the end of the follow-up period.

		CS *N* = 47	DM *N* = 31	CS + DM N = 17	P
Variables recorded at diagnosis	Age *(years)*	11 (10–13)[a]	10 (8–13)[a]	10 (9–13)[a]	0.128
	Weight *(kg)*	6.5 (5.0–11.5)[a]	8.1 (6.4–13.6)[a]	6.8 (4.8–9.3)[a]	0.256
	ALT *(10-110 U/L)*	186 (99–313)[a]	140 (75–303)[a]	172 (100–244)[a]	0.838
	ALKP *(23-212 U/L)*	444 (181–1174)[a]	429 (132–1496)[a]	838 (173–1498)[a]	0.756
	Glucose *(74-143 mg/dL)*	103 (89–122)[a]	444 (334–550)[b]	452 (135–600)[b]	<0.005
Last variables recorded	Age *(years)*	12 (12–14)[a]	12 (9–15)[a]	11(10–13)[a]	0.120
	Weight *(kg)*	6.7 (4.4–10.4)[a]	8.7 (6.0–22.4)[a]	5.8 (4.8–9.2)[a]	0.122
	ALT *(10-110 U/L)*	159 (68–260)[a]	57 (26–142)[a]	169 (104–263)[a]	0.093
	ALKP *(23-212 U/L)*	141 (81–813)[a]	350 (205–431)[a]	400 (312–535)[a]	0.201
	Glucose *(74-143 mg/dL)*	87 (80–107)[a]	278 (166–396)[b]	391 (169–533)[b]	<0.005

Abbreviations: ALT, alanine aminotransferase; ALKP, alkaline phosphatase; DM, diabetes mellitus; CS naturally-occurring Cushing's syndrome; CS+ DM, concurrent Cushing's syndrome and diabetes mellitus.
Data are represented as median (interquartile range). Different superscripts indicate significant differences ($P < 0.05$).
At diagnosis weight was recorded in 47 dogs with CS, 22 dogs with DM and 16 dogs with CS + DM. ALT was recorded in 40 dogs with CS, 7 with DM and 9 with CS + DM. ALKP was recorded in 36 dogs with CS, 7 with DM and 9 with CS + DM. Glucose was recorded in 45 dogs with CS, 22 with DM and 16 with CS + DM. Other dogs did not have a blood glucose concentration recorded because some dogs were remitted with the diagnosis of diabetes, treated with insulin, and presenting hyperglycemia, but the initial blood glucose at diagnosis was not available in the clinical record.
At the end, Weight was recorded in 40 dogs with CS, 26 dogs with DM and 15 dogs with CS + DM. ALT was recorded in 32 dogs with CS, 14 dogs with DM and 8 dogs with CS + DM. ALKP was recorded in 31 dogs with CS, 15 dogs with DM and 8 dogs with CS + DM. Glucose was recorded in 31 dogs with CS, 23 with DM and 14 with CS + DM. (Final glucose of dogs with DM or concurrent CS and DM, should be interpreted carefully since dogs were treated with insulin and glucose were recorded at different moments of the day).

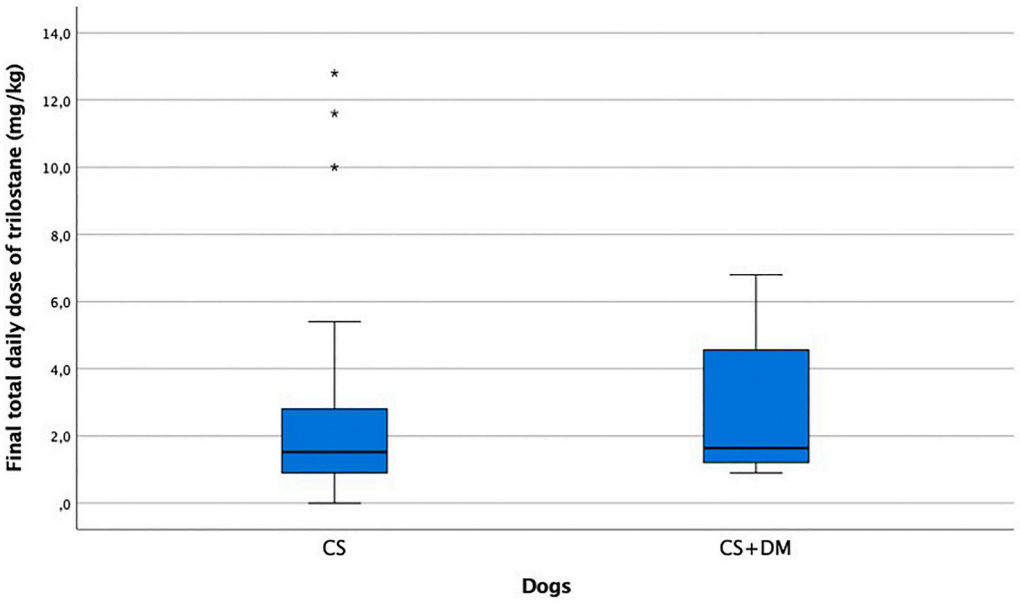

Fig. 1. Box plots of final trilostane total daily dose (mg/kg) in dogs with CS and in dogs with concurrent CS and DM. The solid line in the box represents the median, the outer lines of the box represent the IQR (25th, 75th percentile), and whiskers above and below represent maximum and minimum values respectively, with the exceptions of extreme outliers (asterisks).
Abbreviations: DM, diabetes mellitus; CS, naturally-occurring Cushing's syndrome.

3.1. Survival analysis

At the time of the survival analysis 30 dogs (17 dogs with CS, 9 dogs with concurrent CS and DM and 4 dogs with DM) had been euthanized or died. In some dogs cause of death was unknown due to missing data in clinical records, or the owner or veterinarian could not be contacted, or they were unable to give explanation.

Cause of death among dogs with CS were unknown (six dogs), pancreatitis (one dog) and intraoperative death due to choledochotomy (one dog). Euthanasia was performed in nine dogs with CS for the following reasons: neurological disorders without a definitive diagnosis reached (three dogs), mast cell tumor (two dogs), neurological signs due to invasive pituitary adenoma (one dog), congestive heart failure (one dog), chronic kidney disease (one dog), uncontrolled CS (one dog).

In dogs with concurrent CS and DM, four dogs died for unknown reasons, one dog died because of pancreatitis and diabetic ketoacidosis, and other dog died due to hypoglycemia. In addition, euthanasia was performed in three dogs, one of them presented paraparesis due to calcification of iliac arteries and had concurrent epilepsy, one dog had pancreatitis and the other dog had uncontrolled CS and DM.

Among dogs with DM, two dogs died due to chronic kidney disease, one for unknown reasons, and one dog received euthanasia due to uncontrolled DM.

No risk factors for death were found in dogs with CS, DM or dogs with concurrent CS and DM; results of cox regression analysis are shown in Table 4.

Median survival time in dogs that only presented CS was 1245 days [95% confidence interval (CI), 809–1681]. The survival time was significantly lower in dogs with CS compared to dogs that only presented DM ($P = 0.002$).

The median survival time of dogs that only presented DM was not reached; although it was significantly longer than the median survival

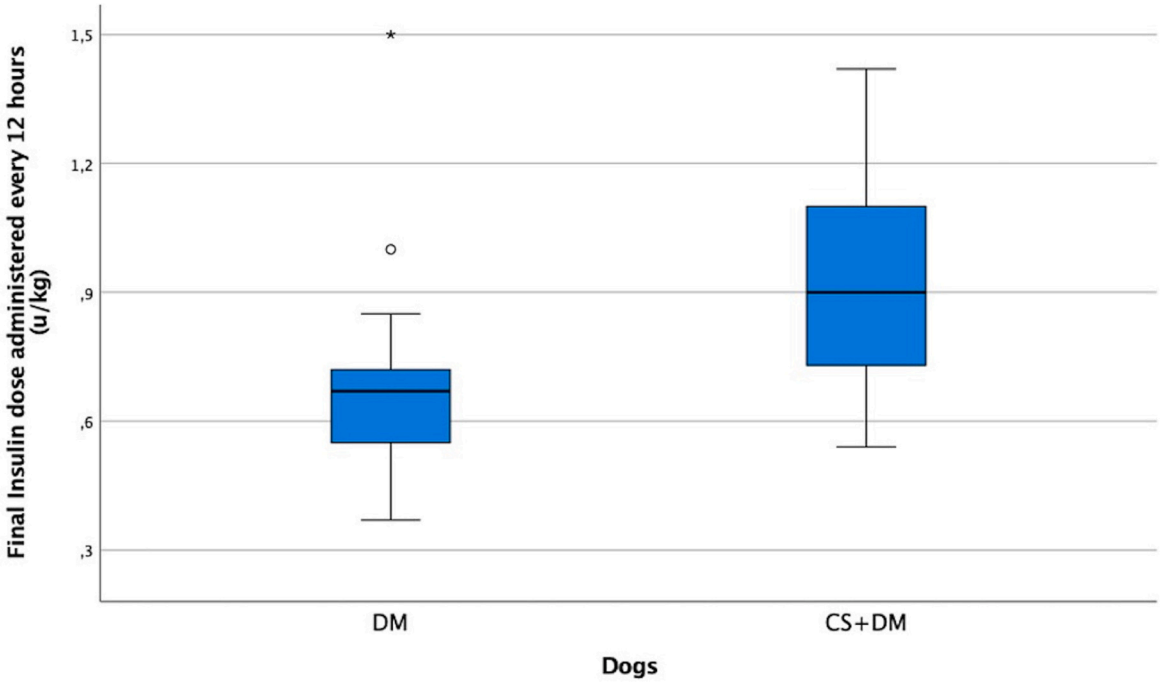

Fig. 2. Box plots for final insulin dose (u/kg) every 12-h in dogs with DM and dogs with concurrent CS and DM. The solid line in the box represents the median, the outer lines of the box represent the IQR (25th, 75th percentile), and whiskers above and below represent maximum and minimum values respectively, with the exceptions of outliers (circles) and extreme outliers (asterisks).
Abbreviations: DM, diabetes mellitus; CS, naturally-occurring Cushing's syndrome.

Table 4
Univariate Cox proportional hazard regression analysis.

	Variables	Risk ratio	95% IC	P
	Sex	0.96	0.32–3.26	0.958
CS	Weight (kg)	0.99	0.95–1.04	0.776
	Age (years)	1.33	0.99–1.79	0.058
	Cortisol 0 h (mg/dL)	0.97	0.88–1.08	0.522
	Cortisol 1 h (mg/dL)	0.96	0.88–1.04	0.237
	ALKP (U/L)	1.00	0.99–1.00	0.901
	ALT (U/L)	1.00	1.00–1.00	0.809
	Cholesterol (mg/dL)	1.00	0.99–1.01	0.330
	Triglycerides (mg/dL)	1.00	0.99–1.01	0.565
	Glucose (mg/dL)	1.01	0.99–1.04	0.410
	Sex	0.78	0.07–9.43	0.858
DM	Weight (kg)	0.02	0.95–1.09	0.567
	Age (years)	1.31	0.88–1.95	0.157
	ALKP (U/L)	0.99	0.99–1.02	0.059
	ALT (U/L)	0.99	0.98–1.02	0.931
	Glucose (mg/dL)	1.00	0.99–1.01	0.974
	Sex	0.97	0.25–3.70	0.96
CS + DM	Weight (kg)	1.05	0.78–1.43	0.74
	Age (years)	0.99	0.65–1.50	0.95
	Cortisol 0 h (mg/dL)	1.05	0.89–1.25	0.61
	Cortisol 1 h (mg/dL)	1.02	0.96–1.08	0.53
	ALKP (U/L)	0.99	0.98–1.01	0.33
	ALT (U/L)	1.00	0.99–1.01	0.84
	Cholesterol (mg/dL)	1.00	0.99–1.01	0.99
	Triglycerides (mg/dL)	0.98	0.91–1.06	0.61
	Glucose (mg/dL)	1.00	0.99–1.01	0.18

*Cholesterol and triglycerides could not be analyzed in dogs with DM due to the small sample size.
Abbreviations: DM, diabetes mellitus; CS, naturally-occurring Cushing's syndrome; CS + DM, concurrent naturally-occurring Cushing's syndrome and diabetes mellitus.

time of dogs with concurrent DM an CS (892 days, [95% CI, 546–1238]; P = 0.002). In contrast, survival time between dogs that only presented CS and dogs with concurrent CS and DM was not significantly different (1245 [95% CI, 809–1681] vs 892 [95% CI, 546–1238] days; *P* = 0.152). Kaplan-Meier survival functions are shown in Figs. 3.

4. Discussion

The insulin antagonism effect caused by CS is a well-recognized cause of diabetes in dogs. However, the life expectancy or management of dogs with concurrent CS and DM has been scarcely evaluated and further investigation could improve the quality of life and life expectancy of dogs with concurrent disorders (Hess, 2010; Nelson, 2015).

This study showed that dogs with concurrent CS and DM had a shorter median survival time (892 days) compared to those that only presented DM, similarly to a recent study that showed that CS was associated with shorter survival time in dogs with DM (645 vs 993 days) (Tardo et al., 2019).

Dogs that only presented CS did not survive significantly longer than dogs with concurrent CS and DM (1245 vs 892 days). By contrast, in a previous study, dogs with concurrent CS and DM had a shorter survival time compared to dogs with CS (Miceli et al., 2017). However, the survival time of dogs with CS reported in the mentioned study [28 months (approximately 850 days)] seems lower than the survival time of the dogs with CS included in the present study (1245 days). Inclusion of dogs with different type of CS could have influenced differences in survival time, although in this case different types of treatments could probably explain the differences in survival time between the two studies. In our study dogs with CS were treated with trilostane, while in this earlier study dogs with CS were treated with retinoic acid and cabergoline or they underwent adrenalectomy, depending on the type of the CS (pituitary dependent or adrenal dependent, respectively). Cabergoline is a dopamine D2 receptor agonist and, in one study, it was reported to be useful to control CS in only 42.5% of dogs with pituitary dependent CS (Castillo et al., 2008); meanwhile acid retinoic treatment has been scarcely used and evaluated (Castillo et al., 2006). The median survival time reported in dogs with pituitary dependent CS treated with retinoic acid (approximately 450 days) (Castillo et al., 2006) seems lower compared to the survival time of dogs with CS treated with

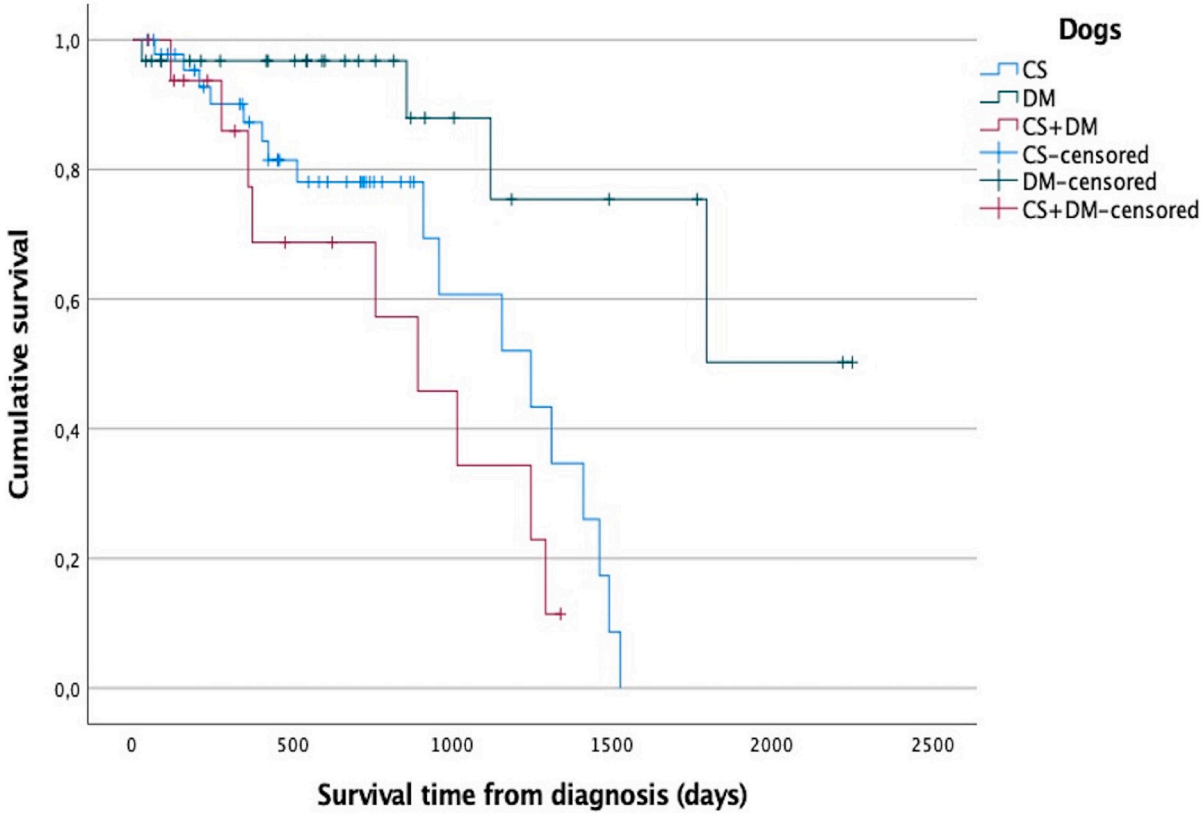

Fig. 3. Kaplan-Meier survival curve for dogs with dogs with diabetes mellitus, dogs with naturally-occurring Cushing's syndrome and dogs with concurrent naturally-occurring Cushing's syndrome and diabetes mellitus. Dogs alive at the completion of the study and those lost to follow-up were censored.

trilostane in this (1155 days) and two other studies which included a population of dogs with pituitary dependent CS and reported a mean survival time of 930 days and a median survival time of 998 days respectively (Pérez-Alenza et al., 2006; García-San José et al., 2022).

Survival negative prognostic factors were not found in the present study. Few variables were considered for this analysis (age, sex, ALT, ALKP, cholesterol, triglycerides, baseline cortisol and glucose). These findings are at odds with those of a recent study that found age as a risk predictor factor of death in a population of dogs with pituitary dependent CS (García - San José et al., 2022). The lower sample size of our study might have underpower results of the cox regression analysis.

Only limited information is available regarding the management of dogs with concurrent DM and CS. Results of the present study has shown that the final trilostane dose was not statistically different between dogs that only presented CS and dogs with concurrent CS and DM. By contrast, dogs with concurrent CS and DM required higher final doses of insulin compared to dogs that only presented DM (median insulin dose 0.90 u/kg vs 0.67 u/kg) despite trilostane treatment. Similarly, one retrospective study evaluated insulin dose in dogs with DM and CS before and after treatment with trilostane. In these dogs, insulin (Caninsulin®) requirements were high (median dose 1.1 u/kg/12 h) before treatment of CS and remained high (1.5 u/kg/12 h) after trilostane treatment (McLauchlan et al., 2010). Higher requirements of insulin dose in dogs with DM and concurrent CS could be explained by uncomplete resolution of hypercortisolism due to the limited duration of trilostane action and the variability of the effect of trilostane from dog to dog (Arenas-Bermejo et al., 2020). These factors could lead to increased cortisol concentrations at time intervals during the day, which can impair insulin action and make diabetes mellitus more difficult to control.

In dogs with DM and occult CS, CS could be suspected when insulin resistance is present and clinical signs such as polyuria/polydipsia and polyphagia do not resolve or signs that are characteristic of CS (truncal or tail alopecia) become apparent (Fleeman and Barrett, 2023). Once hormonal tests are performed and CS is confirmed, it might be expected that trilostane treatment would improve insulin efficacy and result in a decreased insulin requirement. In the present study, insulin dose was higher in dogs with concurrent CS and DM compared to dogs that only have DM, and insulin requirements remained high despite trilostane treatment in dogs with concurrent CS and DM.

An early recognition or diagnosis of both DM and CS might allow a better control of these disorders. Nonetheless, CS and DM are disorders with similar laboratory and clinical presentation, and therefore, sometimes one of these diseases could be overlooked in dogs presenting both disorders concurrently. As previously reported, this study found that in most dogs with concurrent CS and DM these disorders are not diagnosed at the same time (Miceli et al., 2017). In our study, DM was diagnosed first in a greater proportion of dogs (47.1%). This situation could be paradoxical since CS is a predisposing cause for DM but DM is not a predisposing cause for CS. However, this could be related to an easier recognition of the symptoms of DM and easier confirmation of the diagnosis of DM. Furthermore, confirmation of CS through hormonal tests might be delayed in unstable diabetic dogs.

Some limitations are acknowledged in this study. First, the retrospective nature of the study, which could lead to bias from missing data. In addition, survival time also depends on the type of CS (Helm et al., 2011; Arenas et al., 2014; García-San José et al., 2022), and this retrospective analysis did not allow to distinguish between type of CS. Moreover, dogs with smaller pituitary adenomas can live longer than those presenting invasive pituitary macroadenomas or carcinoadenoma (Kent et al., 2007); and evaluation of tumor size was not available in this study. Other limitation could be the small number of dogs included as dogs with concurrent CS and DM, which may underpowered results of this study. Furthermore, prospective studies and evaluation of well-

controlled versus uncontrolled dogs with CS and DM are warranted for a better evaluation of insulin requirements in dogs treated with trilostane, as well as studies evaluating dogs with DM separately in the presence of adrenal dependent CS or in the presence of pituitary CS.

5. Conclusions

Insulin requirements are higher in dogs with concurrent CS and DM compared to dogs that only present DM. Despite trilostane treatment, insulin requirements remain higher in dogs with concurrent CS and DM compared to dogs with only DM. Furthermore, dogs with concurrent disorders (CS and DM) have a shorter survival time compared to dogs that only develop DM. In contrast, dogs with concurrent CS and DM do not have a shorter survival time compared to dogs that only develop CS.

Animal welfare statement

Ethics approval was not needed for this study.

Declaration of Competing Interest

The authors declare not conflict of interests.

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