

Evaluation of individual low-dose dexamethasone suppression test patterns in naturally occurring hyperadrenocorticism in dogs

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Background: Dogs with hyperadrenocorticism (HAC) may be more mildly affected at the time of diagnosis today, which could influence the prevalence of associated clinical and clinicopathological abnormalities and diagnostic test performance. Different low-dose dexamethasone suppression test (LDDST) result patterns have not been evaluated individually.

Objectives: To assess the current features of HAC and evaluate if the diagnostic test performance of individual LDDST result patterns differ.

Animals: One hundred and twenty-three dogs undergoing investigation for HAC.

Methods: Retrospective evaluation of dogs in which a LDDST was performed and HAC confirmed or excluded by alternative means. Cases with basal cortisol concentrations (t_0) < 1 µg/dL were excluded. Each LDDST result was classified as (a) complete suppression (t_3 and t_8 < 1 µg/dL), (b) lack of suppression (t_3 and t_8 > 1 µg/dL and both > 50% t_0), (c) partial suppression (t_3 and t_8 > 1 µg/dL but either < 50% t_0), (d) escape (t_8 > 1 µg/dL and t_3 < 1 µg/dL) or (e) inverse (t_3 > 1 µg/dL and t_8 < 1 µg/dL) pattern.

Results: Fifty-nine (48%) dogs were diagnosed with HAC and 64 (52%) with non-adrenal illness. Hyperadrenocorticism cases had similar clinicopathological abnormalities compared to previous reports. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) (95% confidence interval [CI]) of the LDDST for diagnosing HAC were 96.6 (91.9–100)%, 67.2 (55.7–78.7)%, 73.1 (63.2–82.9)%, and 95.6 (89.5–100)%, respectively. Lack of suppression pattern had the highest PPV (93.9 [85.8–100]%) followed by the partial suppression pattern (67.9 [50.6–85.2]%) and escape or inverse pattern (36.8 [15.1–58.5]%).

Conclusions and Clinical Importance: A lack of suppression LDDST pattern has the highest PPV for diagnosing HAC followed by a partial suppression pattern. By contrast, the escape or inverse pattern provided limited support of HAC.

KEYWORDS

adrenal cortex, adrenal function test, canine, Cushing's syndrome, endocrinology, pituitary

Abbreviations: ACTH, adrenocorticotropic hormone; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AT, adrenal tumor; CI, confidence interval; CT, computed tomography; DGGR, 1,2-o-dilauryl-rac-glycero-3-glutaric acid-(6'-methylresorufin) ester; HAC, hyperadrenocorticism; LDDST, low-dose dexamethasone suppression test; MRI, magnetic resonance imaging; NAI, non-adrenal illness; NPV, negative predictive value; PDH, pituitary-dependent hyperadrenocorticism; PPV, positive predictive value; SD, standard deviation; t_0 , cortisol concentration immediately before dexamethasone administration; t_3 , cortisol concentration 3 or 4 hours after dexamethasone administration; t_8 , cortisol concentration 8 hours after dexamethasone administration.

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1 | INTRODUCTION

A suspicion of hyperadrenocorticism (HAC) in dogs typically is based on history and physical examination findings and can be further supported by several routine laboratory abnormalities.¹ Among the diagnostic tests recommended to help confirm a diagnosis, the adrenocorticotrophic hormone (ACTH) stimulation test and the low-dose dexamethasone suppression test (LDDST) are commonly used in practice.² Neither of these tests is wholly accurate in confirming the diagnosis in animals with HAC, nor in excluding it in dogs presenting with non-adrenal illness (NAI). The ACTH stimulation test reportedly has low sensitivity but high specificity for diagnosis of HAC and performs particularly poorly in dogs with functional adrenal tumors (AT).³⁻⁸ The sensitivity and specificity of the LDDST for diagnosis of HAC are reported as being 85%-100% and 44%-73%, respectively.^{3,4,8-14} Because the LDDST lacks specificity, it is only recommended in dogs in which there is a strong indication to pursue a diagnosis of HAC to maximize the positive predictive value (PPV) of the test. Where specifically evaluated, the PPV of the LDDST has been reported to be as low as 38% and as high as 92%.^{4,8,9} However, all of these studies were published over 20 years ago and only 1 study evaluated PPV in a population of dogs in which HAC was suspected clinically.⁹ Today, in light of increased awareness of HAC and enhanced knowledge of and experience with diagnostic test performance, it has been suggested that the population of dogs currently being evaluated for HAC is different.^{1,15} Consequently, the PPV of the LDDST also may be different, compared to that previously reported.

Several different LDDST result patterns have been described,^{14,16} including those with or without some degree of cortisol suppression allowing identification of pituitary-dependent hyperadrenocorticism (PDH) in some cases. Whether such individual patterns are associated with more or less false-positive results has not yet been evaluated. Recently, a LDDST pattern with a 4-hour cortisol concentration above and an 8-hour cortisol concentration below the cut-off, referred to as the inverse pattern, has been described in 5 dogs with PDH.¹⁰ Historically, this pattern would not have been considered consistent with HAC.^{3,4,8,9,11-14} More recently, it has been considered highly suspicious for HAC.¹ This pattern also was reported in 2 dogs with NAI in the original report.¹⁰ Therefore, it is unclear if such an inverse LDDST pattern should or should not increase the level of confidence for diagnosing HAC.

The aim of our study was to elucidate if dogs with HAC have milder disease today and to further evaluate the LDDST for its diagnosis. It was hypothesized that some individual patterns have higher PPV compared to the historical interpretation of the LDDST in a population of dogs suspected to have HAC.

2 | MATERIALS AND METHODS

2.1 | Case selection and review

The database of the Diagnostic Endocrine Laboratory of the University College Dublin Veterinary Hospital was retrospectively searched for

dogs that underwent a LDDST between January 2007 and December 2016. A LDDST is routinely performed at our hospital as part of the investigation for HAC. Dogs were considered eligible for the study if the LDDST result included a cortisol concentration immediately before dexamethasone administration (t_0), and 3 or 4 hours (either referred to as t_3) and 8 hours (t_8) after dexamethasone administration and if the case was investigated on-site. Non-canine species and cases with a t_0 cortisol concentration $< 1 \mu\text{g/dL}$ were excluded from further evaluation. If cases were admitted for investigation more than once during the study period, only the most recent visit was included.

The medical records from the eligible cases were retrospectively reviewed by 2 authors (C. Forde and M. Bennaim). Cases receiving a medication expected to alter adrenal function testing results at the time of diagnosis (eg, glucocorticoids, mitotane, trilostane, ketoconazole) were excluded from further evaluation. Cases were considered for inclusion in the study if the records reflected the presence of 1 common clinical sign of HAC, 2 uncommon clinical signs of HAC, or 1 uncommon clinical sign of HAC associated with increased alkaline phosphatase (ALP) or alanine aminotransferase (ALT) activities and hypercholesterolemia, as previously reported.¹ Data collected included signalment, clinical findings before diagnosis, results from hematology, biochemistry, urinalysis, urine protein : creatinine ratio, bile acid stimulation test, LDDST (t_0 , t_3 , and t_8 cortisol concentrations), ACTH stimulation test results, ultrasonography or advanced imaging results, final diagnosis, response to treatment for HAC (if applicable), progression of clinical signs, or some combination of these. A manual thrombocyte count was chosen over the automated count, if available. If adequate follow-up regarding treatment response or progression was not available in the medical records, it was requested from owners, veterinarians or both by a telephone conversation with 1 of the authors (M. Bennaim).

Cases were classified as having NAI if 1 of the following criteria was fulfilled: alternative diagnosis for the problem(s) investigated, spontaneous resolution of clinical signs of HAC, or follow-up of at least 1 year available during which (1) the dog did not receive any treatment for HAC (eg, mitotane, trilostane, or adrenalectomy) and (2) the clinical signs that prompted the investigation of HAC did not progress or additional clinical signs of HAC did not develop. Cases were classified as having HAC if a response to treatment for HAC (ie, mitotane, trilostane, or adrenalectomy) could be documented or if postmortem examination identified pituitary adenoma or adrenocortical neoplasia with contralateral adrenal atrophy. Medical records of cases that could not be classified as NAI based on the above criteria or that never received treatment for HAC and for which a postmortem examination was not performed were reviewed independently by 2 board-certified internists (C. T. Mooney and R. E. Shiel) and included only if there was mutual agreement on a final diagnosis of HAC. This review involved complete evaluation of all information (history, clinical and clinicopathological data, diagnostic imaging results, ancillary diagnostic tests to eliminate other disorders, ACTH stimulation test results and photographs, if available, without specific reference to the LDDST results).

Cases in the HAC group were defined as PDH if any of the following criteria were met: endogenous ACTH concentration $> 5 \text{ pg/mL}$

(ELISA validated for dogs [sensitivity of 0.22 pg/mL, detection limit of 5 pg/mL, intra-assay CV of less than 6.7% and interassay CV of less than 7.1%] Nationwide Laboratories, UK),¹⁷ pituitary enlargement on magnetic resonance imaging (MRI) or computed tomography (CT) or bilaterally normal to enlarged adrenal glands on abdominal ultrasonography. All adrenal glands > 4 cm were considered neoplastic.¹⁸ Functional AT was diagnosed if any of the following criteria were met: endogenous ACTH concentration < 5 pg/mL,¹⁷ an adrenal mass with a maximal dorsoventral thickness of the contralateral gland \leq 5 mm¹⁸ or histopathological confirmation of adrenocortical neoplasia associated with resolution of clinical signs of HAC after adrenalectomy. If an adrenal mass was identified but the criteria for functional AT were not fulfilled, such dogs were grouped separately.

2.2 | Cortisol assays

Cortisol concentration was measured in heparinized samples using a solid-phase, competitive chemiluminescent immunoassay previously validated^{19,20} in dogs (Immulite 1000 [Immulite 1000 Cortisol, Siemens Healthcare Diagnostics Ltd., Gwynedd, UK] and Immulite 2000 analyzer [Immulite 2000 Cortisol, Siemens Healthcare Diagnostics Ltd., Gwynedd, UK], respectively, before and after 2012). All samples, calibrators and quality-control samples were run according to the manufacturer's instructions. The limit of detection of the assay was 1 μ g/dL.

2.3 | Adrenal function testing

For ACTH stimulation testing, plasma cortisol concentrations were measured immediately before and 1 hour after administration of a standard IM dose of 125 μ g (for dogs < 5 kg) or 250 μ g (for dogs > 5 kg) synthetic ACTH (Tetracosactide, Synacthen; Alliance Pharmaceuticals, Chippenham, UK). A positive ACTH stimulation test was defined as a post-ACTH cortisol concentration > 21.7 μ g/dL.

For the LDDST, plasma cortisol concentrations were measured immediately before, 3 or 4 hours and 8 hours after IV administration of 0.015 mg/kg of dexamethasone. To encourage acclimatization and minimize stress, the LDDST routinely was performed after at least 1 day of hospitalization, with no other procedures scheduled during the test. Dogs were not fasted during the test.

A positive LDDST result was defined as either or both a t_3 or t_8 cortisol concentration > 1 μ g/dL (therefore, including the inverse pattern as positive). For the purpose of our study, each LDDST result was retrospectively classified as complete suppression (both t_3 and t_8 cortisol concentrations < 1 μ g/dL), lack of suppression (both t_3 and t_8 cortisol concentrations > 1 μ g/dL and > 50% t_0 cortisol concentration), partial suppression (t_3 and t_8 cortisol concentrations > 1 μ g/dL but either or both < 50% t_0), escape (t_3 cortisol concentration < 1 μ g/dL and t_8 cortisol concentration > 1 μ g/dL), or inverse (t_3 cortisol concentration > 1 μ g/dL and t_8 cortisol concentration < 1 μ g/dL) pattern.

2.4 | Data analysis

Normality of the data was assessed by the Shapiro-Wilk test. Descriptive statistics were determined and expressed as mean (\pm standard

deviation [SD]) for normally distributed variables and median (range) for non-normally distributed variables. For age and clinicopathological data, a Mann-Whitney *U* test was used if not normally distributed and a student *t* test was used if normally distributed.

Sensitivity, specificity, PPV, negative predictive values (NPV), and diagnostic accuracy of the LDDST and PPV of the different patterns (lack of suppression, partial suppression, escape, and inverse) and their associated 95% confidence intervals (CIs) were calculated using online statistical software.²¹

The sex distribution, frequency of individual clinical signs, and proportion of dogs within different urine specific gravity categories were compared between dogs with HAC and NAI using the chi-square test of independence if the expected values were > 5 and the Fisher's exact test if the expected values were \leq 5. These tests also were used to compare the PPV of the different LDDST patterns. All analyses were performed using commercially available software (IBM SPSS v. 20 for Mac OS X; IBM Corporation, New York, NY) and *P* values < .05 were considered significant.

3 | RESULTS

3.1 | Study population

Four-hundred and twenty-seven cases for which investigations included a LDDST initially were identified. After review of the medical records for 135 eligible cases (Figure 1), 123 cases, including 59 dogs diagnosed with HAC and 64 dogs diagnosed with NAI, were included in the study, reflecting a disease prevalence of 48.0%. A diagnosis of HAC was supported by documented improvement with treatment in 43 cases over a follow-up period of 180 (range, 8–2049) days, post-mortem examination identifying a pituitary adenoma in 3 cases and independent review of the medical records by 2 board-certified internists in 13 cases. A diagnosis of NAI was supported by an alternative diagnosis in 58 cases, spontaneous resolution of clinical signs in 3 cases, and absence of progression of clinical signs or onset of additional clinical signs over a follow-up period > 1 year in 3 cases.

Mean \pm SD and median (range) age at diagnosis were 9.7 (\pm 2.4) and 9.7 (5.1–14.6) years in the HAC group and the median (range) in the NAI group was 9.8 (3.2–14.2) years. The HAC and NAI groups included 36 female neutered, 4 female intact, 11 male neutered, 8 male intact and 32 female neutered, 6 female intact, 20 male neutered, 6 male intact, respectively. Age (*P* = .33) and sex (*P* = .34) did not differ significantly between the 2 groups. Breeds in the HAC group included crossbreed (*n* = 12), Boxer (*n* = 5), Springer Spaniel (*n* = 4), Bichon Frise (*n* = 4), Cavalier King Charles Spaniel (*n* = 4), Golden Retriever (*n* = 3), Yorkshire Terrier (*n* = 3), Border Terrier (*n* = 2), Cairn Terrier (*n* = 2), Greyhound (*n* = 2), Labrador Retriever (*n* = 2), Scottish Terrier (*n* = 2), Wheaten Terrier (*n* = 2), and 1 each of Beagle, Boston Terrier, Briard, Cocker Spaniel, French Bulldog, German Shepherd, Rhodesian Ridgeback, Jack Russell Terrier, Maltese, Miniature Schnauzer, Rottweiler, and Staffordshire Bull Terrier. Breeds in the NAI group included crossbreed (*n* = 11), Boxer (*n* = 5), Cavalier King Charles Spaniel (*n* = 6), Labrador Retriever (*n* = 6), Yorkshire Terrier (*n* = 5), West Highland

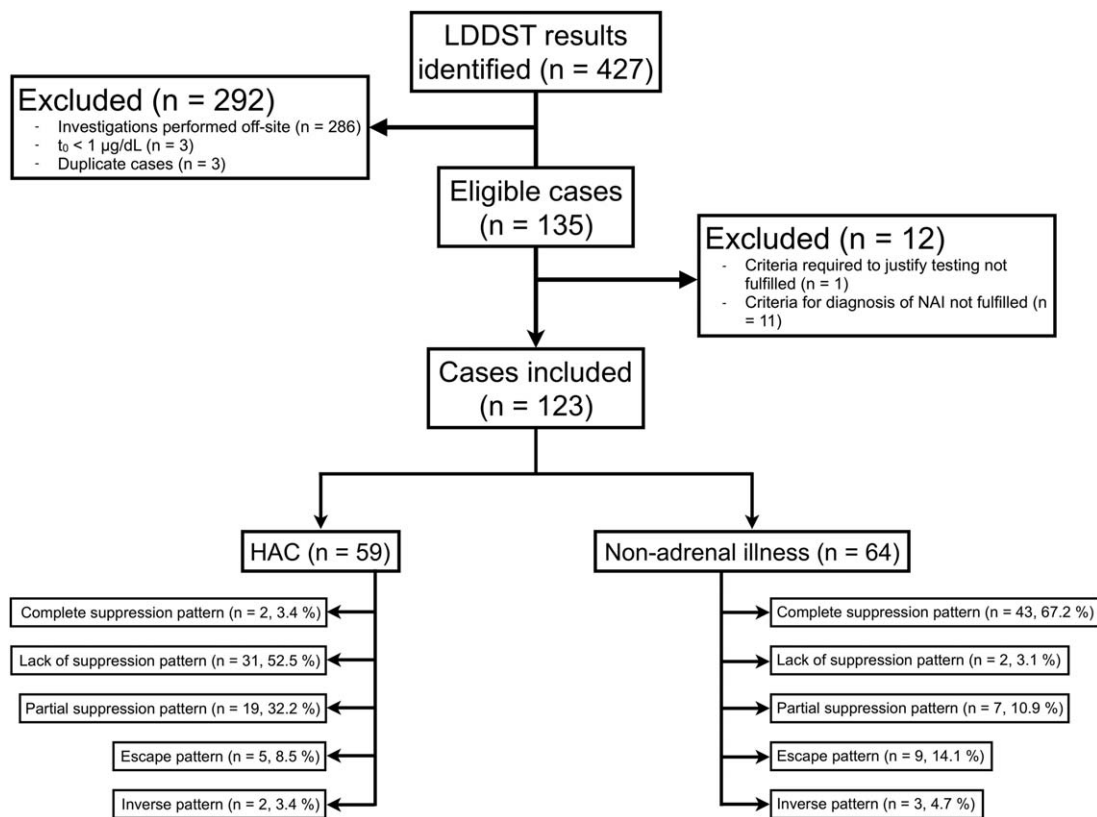


FIGURE 1 A flow chart showing the selection of cases included in the study and the repartition of the different low-dose dexamethasone suppression test patterns in each group

White Terrier (n = 4), Shih Tzu (n = 2), Jack Russell Terrier (n = 2), Golden Retriever (n = 2), Pomeranian (n = 2), Scottish Terrier (n = 2), Tibetan Terrier (n = 2) and 1 each of Shetland Sheepdog, Cocker Spaniel, Cairn Terrier, Welsh Corgi, Dachshund, Dalmatian, Dobermann, German Shepherd, Irish Wolfhound, Kerry Blue Terrier, Maltese, Parson Russell Terrier, Poodle, Samoyed, and Wire Haired Fox Terrier.

3.2 | Clinical and clinicopathological data

Complete clinical data were available for all dogs included in the study and results are presented in Table 1. The most frequent clinical signs were polyuria (94.9%), polydipsia (91.5%), lethargy (64.4%), and abdominal enlargement (52.5%) in dogs with HAC, and polydipsia (78.1%), polyuria (65.6%), lethargy (50%), and weight gain (29.7%) in dogs with NAI. Histopathology was performed in 2 of the 4 dogs with HAC in which calcinosis cutis was suspected and confirmed the suspicion. The prevalence of polyuria ($P < .001$), polydipsia ($P = .040$), abdominal enlargement ($P < .001$), alopecia ($P = .024$), excessive panting ($P = .015$), and muscle wasting ($P = .002$) was significantly higher in dogs diagnosed with HAC.

Results for hematology and biochemistry were available for 58 of 59 (98.3%) dogs with HAC and 61 of 64 (95.3%) dogs with NAI. In 1 dog with NAI for which the results of hematology were available, the thrombocyte count was not included. The lipase activity and glucose concentration could not be retrieved from, respectively, 2 (with HAC) and 3 (2 with HAC and 1 with NAI) dogs in which the remainder of the

biochemistry was available. A bile acid stimulation test was performed in 16 dogs with HAC and 19 dogs with NAI. A fasting bile acids concentration alone was measured in 1 further dog with HAC. Results for hematology, biochemistry, and bile acids concentration are presented in Table 2. Lymphocyte ($P < .001$) and eosinophil ($P < .001$) counts and serum creatinine concentration ($P < .001$) were significantly lower whereas neutrophil ($P = .007$) and thrombocyte ($P = .002$) counts, ALP ($P < .001$), ALT ($P < .001$), and lipase ($P < .001$) activities and serum phosphorus ($P < .001$) and cholesterol ($P < .001$) concentrations were significantly higher in dogs with HAC.

Urinalysis results were retrieved from 56 of 59 (94.9%) dogs with HAC and 59 of 64 (92.2%) dogs with NAI. In dogs with HAC and NAI, urine specific gravity was between 1.000 and 1.007 in 20 (35.7%) and 10 (16.9%) dogs ($P = .022$), between 1.008 and 1.012 in 16 (28.6%) and 9 (15.2%) dogs ($P = .083$), between 1.013 and 1.030 in 19 (33.9%) and 23 (39.0%) dogs ($P = .57$) and > 1.030 in 1 (3.6%) and 17 (28.8%) dogs ($P < .001$), respectively. The 1 dog with HAC and urine specific gravity > 1.030 had concurrent glucosuria. In total, glucosuria was identified on urine dipstick analysis in 5 (8.9%) dogs with HAC and 5 (8.5%) dogs with NAI ($P = 1$). Among dogs in which glucosuria was identified, the blood glucose concentration was < 153 mg/dL in 1 dog with NAI and 3 dogs with HAC. All of these dogs had glucosuria graded as 1+. The blood glucose concentration was > 345 mg/dL in the remaining glucosuric dogs. Proteinuria was identified on urine dipstick analysis more frequently in dogs with HAC (n = 45 [80.3%]), 5 dogs had trace, 9 had 1+, 26 had 2+, and 5 had 3+, compared to dogs with NAI

TABLE 1 Prevalence of individual clinical signs in dogs included in the study

	HAC group (n = 59)	NAI group (n = 64)	P value
Polyuria (%)	56 (94.9)	42 (65.6)	< .001
Polydipsia (%)	54 (91.5)	50 (78.1)	.040
Lethargy (%)	38 (64.4)	32 (50)	.11
Abdominal enlargement (%)	31 (52.5)	11 (17.2)	< .001
Excessive panting (%)	25 (42.4)	14 (21.9)	.015
Polyphagia (%)	25 (42.4)	14 (21.9)	.015
Alopecia (%)	24 (40.7)	14 (21.9)	.024
Weight gain (%)	20 (33.9)	19 (29.7)	.62
Muscle wastage (%)	16 (27.1)	4 (6.3)	.0020
Weight loss (%)	15 (25.4)	7 (10.9)	.036
Thin skin (%)	12 (20.3)	6 (9.4)	.086
Vomiting (%)	11 (18.6)	14 (21.9)	.66
Diarrhea (%)	7 (11.9)	11 (17.2)	.40
Comedones (%)	6 (10.2)	2 (3.1)	.15
Pruritus (%)	5 (8.5)	8 (12.5)	.47
Calcinosis cutis (%)	4 (6.8)	0 (0)	.050
Inappetence (%)	3 (5.1)	7 (10.9)	.33

HAC, hyperadrenocorticism; NAI, non-adrenal illness.

(n = 36 [61.0%], 5 dogs had trace, 14 had 1+, 7 had 2+, and 10 had 3+; $P = .023$). Urine culture was performed in 51 dogs with HAC and 46 dogs with NAI. Bacterial growth was identified in 9 (17.6%) dogs with HAC and 7 (15.2%) dogs with NAI ($P = .75$). Urine protein : creatinine ratio was determined in 8 dogs with HAC and 8 dogs with NAI. Urine culture was performed in 6, and 6 of these dogs, respectively, and was negative in all. Median (range) urine protein : creatinine ratio was 3.4 (2.4–13) in dogs with HAC and 1.15 (0.16–9) in dogs with NAI ($P = .14$).

Results of ACTH stimulation testing were available in all dogs with HAC and 52 of 64 (81.2%) dogs with NAI. For 1 additional dog with NAI, the interpretation of the results was available but not the actual cortisol concentrations. This dog had a post-ACTH cortisol concentration below that considered consistent with HAC by the laboratory (21.7 $\mu\text{g/dL}$). The mean pre- and post-ACTH cortisol concentrations in dogs with functional ATs were 4.6 (± 1.5) $\mu\text{g/dL}$ and 16.9 (± 13.1) $\mu\text{g/dL}$. The median pre- and post-ACTH cortisol concentrations in dogs with PDH were 5.9 (2–23.3) $\mu\text{g/dL}$ and 30.5 (10.4–> 50) $\mu\text{g/dL}$. In 3 additional dogs with AT and suspected concurrent PDH, the pre- and post-ACTH cortisol concentrations were 3.6 and 17.5, 2.7 and 17.8, and 5.9 and 35.2 $\mu\text{g/dL}$, respectively. The median pre- and post-ACTH cortisol concentrations in dogs with NAI were 3.79 (< 1–17.8) and 15.8 (7.6–32.3) $\mu\text{g/dL}$. The post-ACTH cortisol concentration was > 21.7 $\mu\text{g/dL}$ in 11 of 52 (21.1%) dogs with NAI and 42 of 59 (71.2%) dogs with HAC (including 39 dogs with PDH, 2 dogs with

functional ATs, and 1 dog in which the differentiation could not be made). In 3 dogs with HAC, the response to ACTH administration was considered sub-normal (pre- and post-ACTH cortisol concentration 4.9 and 4.7, 3.8 and 4.2, and 6.2 and 8 $\mu\text{g/dL}$, respectively). All 3 dogs had functional ATs and demonstrated excessive stimulation of 17-hydroxyprogesterone (17-OH progesterone RIA validated for dogs, Nationwide Laboratories, UK) after ACTH administration (7.9 and > 19.8, 10.6 and > 19.8, and 1.9 and 12.2 ng/mL; reference interval, 0.99 and < 2.6 ng/mL, respectively).

3.3 | Low-dose dexamethasone suppression test

A complete suppression pattern, lack of suppression pattern, partial suppression pattern, escape pattern, and inverse pattern was found in 2 (3.4%), 31 (52.5%), 19 (32.2%), 5 (8.5%), and 2 (3.4%) dogs with HAC and in 43 (67.2%), 2 (3.1%), 7 (10.9%), 9 (14.1%), and 3 (4.7%) dogs with NAI, respectively (Figure 1).

The sensitivity, specificity, PPV, NPV, and diagnostic accuracy of the LDDST and the PPV of the different patterns for diagnosing HAC are presented in Table 3. However, because of the low number of escape and inverse patterns and the absence of identifiable difference between their respective PPVs ($P = 1$), these results were combined. The PPV of the lack of suppression pattern was significantly higher than the PPV of the partial suppression pattern ($P = .022$), combined escape and inverse pattern ($P < .001$) and the LDDST overall ($P = .011$). No significant difference was found between the PPV of the partial suppression pattern and the PPV of the LDDST overall ($P = 1$). The PPV of the combined inverse and escape pattern was significantly lower than the PPV of the partial suppression pattern ($P = .023$) and LDDST overall ($P = .006$).

3.4 | Final diagnoses

In the group of 59 dogs with HAC, 47 (79.7%) were diagnosed with PDH and 8 (13.6%) dogs were diagnosed with functional AT. A diagnosis of PDH was made in 45 dogs by abdominal ultrasonography based on adrenal symmetry (n = 42 [89.4%]) or mild asymmetry but with maintenance of normal shape and without evidence of adrenal gland atrophy (n = 3 [6.4%]). One of the latter dogs had an endogenous ACTH concentration of 19.2 pg/mL. The remaining 2 dogs with PDH were diagnosed by brain MRI or abdominal CT. The dogs with functional AT all had marked asymmetric enlargement of the adrenal glands with evidence of atrophy of the smaller gland. Three also had histological confirmation of adrenocortical carcinoma and 3 had endogenous ACTH concentration measured with values consistent with functional AT in all. Three (5.1%) dogs were suspicious of concurrent PDH and AT. Two of these dogs had marked enlargement of 1 adrenal gland (> 4 cm) but with coexistent enlargement of the contralateral gland (maximal dorsoventral thickness of the contralateral gland of 9 and 11 mm, respectively). One of these dogs had a histopathological diagnosis of adrenocortical carcinoma (incompletely removed) affecting the larger gland. In the other dog, 1 adrenal gland was > 4 cm but the contralateral adrenal could not be identified. Endogenous ACTH concentration

TABLE 2 Clinicopathological findings in dogs included in the study

	HAC group				NAI group				P value	
	Number of dogs with abnormal values		Value	n	Number of dogs with abnormal values		Value	n		
	Above RI	Below RI			Above RI	Below RI				
Hematology										
Hematocrit (L/L)	58	0.49 (± 0.06) ^a 0.50 (0.34-0.60) ^a	8 (13.8%)	1 (1.7%)	61	0.48 (0.29-0.58)	5 (8.2%)	3 (4.9%)	0.37-0.55	.170
Lymphocyte count (x 10 ³ /μL)	58	1.09 (± 0.5) ^a 1.09 (0.12-2.3) ^a	0 (0%)	26 (44.8%)	61	1.52 (0.53-4.48)	3 (4.9%)	11 (18%)	1-3.6	<.001
Neutrophil count (x 10 ³ /μL)	58	7.81 (3.66-17.73)	13 (22.4%)	0 (0%)	61	6.13 (2.49-23.10)	4 (6.6%)	2 (3.3%)	3-11.5	.007
Eosinophil count (x 10 ³ /μL)	58	0.05 (0-1.5)	1 (1.7%)	0 (0%)	61	0.19 (0-0.83)	0 (0%)	0 (0%)	0-1.47	<.001
Monocyte count (x 10 ³ /μL)	58	0.61 (0.09-2.83)	1 (1.7%)	0 (0%)	61	0.53 (± 0.28) ^a 0.49 (0.01-1.45) ^a	1 (1.6%)	0 (0%)	0-1.35	.110
Thrombocyte count (x 10 ³ /μL)	58	448 (151-1010)	22 (37.9%)	0 (0%)	60	333 (90-783)	11 (18.3%)	1 (1.7%)	150-500	.002
Biochemistry										
Creatinine (mg/dL)	58	0.8 (0.4-1.8)	1 (1.7%)	0 (0%)	61	0.9 (0.6-2.9)	4 (6.6%)	0 (0%)	0.2-1.4	<.001
Blood urea nitrogen (mg/dL)	58	10.8 (5.0-36.1)	2 (3.4%)	25 (43.1%)	61	12.3 (3.6-48.2)	6 (9.8%)	20 (32.8%)	10.1-24.1	.260
ALP activity	58	19.3 (3.0-146.4)	58 (100%)	0 (0%)	61	4.4 (0.7-126.1)	57 (93.4%)	0 (0%)	0-81	<.001
ALT activity	58	5.1 (0.9-34.4)	55 (94.8%)	0 (0%)	61	1.9 (0.6-20.4)	50 (82%)	0 (0%)	0-81	<.001
Lipase activity (IU/L)	56	72 (0-2433)	27 (48.2%)	0 (0%)	61	35 (10-387)	10 (16.4%)	0 (0%)	0-81	<.001
Glucose (mg/dL)	56	105 (43-750)	11 (19.6%)	1 (1.8%)	60	127 (± 87) ^a 107 (54-502) ^a	13 (21.7%)	0 (0%)	54-117	.520
Phosphorus (mg/dL)	58	4.5 (± 0.9) ^a	6 (10.3%)	1 (1.7%)	61	3.8 (± 1.1) ^a	3 (4.9%)	5 (8.2%)	2.5-5.6	<.001
Cholesterol (mg/dL)	58	336 (192-1085)	48 (82.8%)	0 (0%)	61	259 (107-772)	38 (62.3%)	1 (1.5%)	124-251	<.001
Total calcium (mg/dL)	58	10.8 (± 0.9) ^a	4 (6.9%)	2 (3.4%)	61	11 (± 0.8) ^a	4 (6%)	1 (1.6%)	9.2-12	.150
Potassium (mEq/L)	58	4.2 (2.3-5.9)	1 (1.7%)	9 (15.5%)	61	4.1 (± 0.4) ^a 4.1 (3.2-5.0) ^a	0 (0%)	11 (18%)	3.7-5.8	.410
Pre-prandial bile acids (μmol/L)	17	4.1 (0-19)	2 (11.8%)	0 (0%)	19	2.1 (0-123)	2 (10.5%)	0 (0%)	0-15	.190
Post-prandial bile acids (μmol/L)	16	14.4 (± 11.8) ^a 11.8 (0-39) ^a	6 (37.5%)	0 (0%)	19	5 (0-103)	1 (5.2%)	0 (0%)	0-20	.010

Results are expressed as mean (± SD) or median (range), respectively, for normally and non-normally distributed variables. Median (range) is also reported for normally distributed data if a non-parametric test was used for comparison. Because two different assays for measurement of ALT and ALP activities were used during the study period, results are expressed as a multiple of the upper limit of the reference interval for each, respectively. Lipase activity was measured with the 1,2-o-dilauryl-rac-glycero-3-glutaric acid-(6'-methylresorufin) ester assay.

HAC, hyperadrenocorticism; NAI, non-adrenal illness; ALP, alkaline phosphatase; ALT, alanine aminotransferase; RI, reference interval.

^aNormally distributed data.

TABLE 3 The number of dogs with a positive LDDST result (defined as a cortisol concentration 3 to 4 hours or 8 hours after dexamethasone administration > 1 ug/dL) in each group, sensitivity, specificity, positive predictive value, negative predictive value, diagnostic accuracy of the overall results of the LDDST for diagnosis of HAC

	HAC group (n = 59)	NAI group (n = 64)	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)	Diagnostic accuracy (95% CI)
LDDST	57	21	96.6 (91.9–100)	67.2 (55.7–78.7)	73.1 (63.2–82.9)	95.6 (89.5–100)	81.3 (73.3–87.8)
Lack of suppression pattern	31	2			93.9 (85.8–100)		
Partial suppression pattern	19	7			73.1 (56–90.1)		
Escape pattern	5	9			35.7 (10.6–60.8)		
Inverse pattern	2	3			40 (0–82.9)		
Combined escape and inverse pattern	7	12			36.8 (15.1–58.5)		

CI, confidence interval; HAC, hyperadrenocorticism; LDDST, Low-dose dexamethasone suppression test (overall results); NAI, non-adrenal illness.

was consistent with PDH (31 pg/mL) and the LDDST exhibited a partial suppression pattern. The 1 (1.7%) remaining dog could not be classified in any category. This dog had a lack of suppression pattern on LDDST and asymmetry of the adrenal glands but the smaller gland had a dorso-ventral thickness > 5 mm (5.2 mm). Endogenous ACTH concentration was not measured.

The 29 (48%) complete, escape, inverse, and partial suppression patterns in the HAC groups were identified only in dogs with PDH including 1 with concurrent AT. Among dogs with a lack of suppression pattern, 20 (67.7%) had PDH, 8 (25.8%) had functional AT, and 2 (6.5%) had suspected concurrent PDH and AT. Increasing cortisol concentrations (defined as an increase of > 50% in cortisol concentration between any time without suppression) occurred in 6 dogs categorized within the lack of suppression pattern group. All of these dogs had PDH as identified by symmetric enlargement of both adrenal glands.

An alternative diagnosis was found in 58 dogs with NAI. Two dogs with NAI had a lack of suppression pattern and were diagnosed with central diabetes insipidus and urinary tract infection in combination with *Sarcoptes scabiei* infection, respectively. The dogs with an escape pattern each were diagnosed with primary polydipsia, alopecia X, diabetes mellitus, non-functional adrenocortical carcinoma and pyoderma, calcium oxalate urolithiasis in combination with primary hyperlipidemia, obesity in combination with vacuolar hepatopathy, protein-losing nephropathy in combination with systemic hypertension and obesity, and chronic kidney disease. The remaining dog with an escape pattern had spontaneous resolution of an episode of polyuria and polydipsia. Dogs with a partial suppression pattern were diagnosed with liver dysfunction of unknown cause (n = 2) and 1 each of diabetes mellitus in combination with an incidental mass in 1 adrenal gland, alopecia X, primary hyperlipidemia, urinary tract infection, and cutaneous adverse food reaction. Dogs with an inverse pattern were diagnosed with urinary tract infection and primary hyperlipidemia, pheochromocytoma, and separation anxiety. The remaining dogs were diagnosed with central diabetes insipidus (n = 5), urethral sphincter mechanism incompetence (n = 3), alopecia X (n = 2), urinary tract infection (n = 2), diabetes mellitus (n = 2), incidental mass in 1 adrenal gland (n = 2),

hypothyroidism (n = 2), idiopathic hepatitis (n = 2), and one each of primary polydipsia, pheochromocytoma, diabetes mellitus in combination with an incidental mass in 1 adrenal gland, primary hyperlipidemia, primary hyperparathyroidism, pyogranulomatous dermatitis, vacuolar hepatopathy, urinary tract infection in combination with chronic kidney disease, sudden acquired retinal degeneration, post-inflammatory hyperpigmentation, protein-losing nephropathy and cranial cruciate ligament rupture, protein-losing nephropathy in combination with obesity and atopic dermatitis, pyoderma and cranial cruciate ligament rupture, primary polydipsia in combination with obesity and pyoderma, primary polydipsia in combination with chronic inflammatory enteropathy, chronic bronchitis, congestive heart failure, and an abdominal mass of unknown nature, applying pressure on the bladder. Six additional dogs were included in the NAI group because of spontaneous resolution of their clinical signs (n = 3) and absence of progression of clinical signs or onset of additional clinical signs (n = 3) over a follow-up period of 379, 512, and 1336 days. Notably, 1 of these 3 dogs (concurrently diagnosed with syringomyelia and tracheal collapse) had a complete LDDST suppression pattern but no pituitary mass or pituitary enlargement on MRI, further supporting NAI rather than HAC.

4 | DISCUSSION

The sensitivity of a diagnostic test describes the frequency of positive test results in animals that truly have the disease whereas specificity describes the frequency of negative test results in animals that do not have the disease.²² Generally, no test is 100% sensitive or specific and the prevalence of false-positive and false-negative results will vary from test to test. In reality, sensitivity and specificity provide no information on how reliable a test result is at diagnosing or eliminating a particular disease in any individual animal. Predictive values, on the other hand, better reflect the probability of positive or negative test results truly being accurate. Predictive values are notably determined by the prevalence of the disease in the population under evaluation.²³

The sensitivity of > 90% and specificity of approximately 70% of the LDDST, as found in our study, are similar to the values of 85%–100% and 44–73%, as reported previously, respectively.^{3,4,8,9,11–14} The fact that the diagnostic performance of the LDDST was similar to previous reports is not surprising given that the clinical signs of HAC in our study are similar to those in previous reports (see below). In many previous studies, the inverse pattern would not have been considered positive. Given the report suggesting an association between an inverse pattern and HAC,¹⁰ such a pattern was considered positive in our study for the evaluation of the LDDST overall. The small number of cases with inverse patterns was unlikely to have materially affected the diagnostic test performance of the LDDST overall. There was a high NPV for the LDDST overall in our study, similar to the NPV of the same test in a previous study, calculated for a similar prevalence of HAC.⁹ The high NPV of the LDDST overall supports the value of this test in eliminating a diagnosis of HAC in unaffected dogs. Undoubtedly, the diagnostic performance of the LDDST must be interpreted with caution. Unfortunately, there is no gold standard ante-mortem test for eliminating or diagnosing HAC in dogs with which the LDDST could be compared and the results reported here may not accurately reflect its true diagnostic performance. This limitation is common in studies investigating HAC. However, several steps were taken to minimize the influence of the results of the LDDST in the final diagnosis. The test results were not considered independently. Rather, a diagnosis of HAC was made based on a combination of supportive clinical signs, clinicopathological abnormalities, ACTH stimulation testing, abdominal imaging (as has previously been recommended¹) but without reliance on the LDDST results. Additionally, response to treatment or postmortem examination also was included for the majority (78%) of cases with HAC. In those where such information was not available, the diagnosis of HAC also was supported by specialist review, and for 9 of these 13 cases, by a positive ACTH stimulation test result. For dogs diagnosed with NAI, where a plausible definitive diagnosis was not initially made, additional information (resolution of signs or lack of progression over a year) was sought to ensure limited likelihood of HAC. Such additional information was only required for a minority (< 10%) of NAI cases.

In our study, a lack of suppression pattern was associated with a higher PPV compared to the LDDST results overall and all of the other individual patterns. There were very few (< 3%) false-positive results for the lack of suppression pattern. This finding suggests greater hypothalamic-pituitary-adrenal axis sensitivity to negative glucocorticoid feedback in dogs with NAI compared to dogs with HAC, and consequently, partial or complete suppression of cortisol secretion occurred after dexamethasone administration in almost all dogs with NAI. Thus, in any population with a prevalence of HAC similar to the prevalence of HAC in our study, a lack of suppression pattern is associated with a high probability of HAC.²⁴ This pattern was the most common pattern seen occurring in > 50% of dogs with HAC in our study and this finding reinforces the value of performing a LDDST, despite the lower PPV of this test overall.

The PPV of the partial suppression pattern was not significantly different compared to the LDDST overall and was significantly lower compared to the lack of suppression pattern. Thus, such patterns

cannot be as confidently viewed as the lack of suppression pattern for diagnosing HAC. However, given that this pattern was associated with a greater probability of HAC, it should, therefore, be considered supportive of such a diagnosis. On the other hand, the PPV of the combined escape and inverse patterns was statistically lower compared to the PPV of the LDDST overall and all the other individual patterns. An inverse pattern historically was considered a negative LDDST result^{3,4,8,9,11–13} and has only been considered suggestive of HAC relatively recently.^{1,10} The concern that an escape pattern may not be supportive of HAC has not been raised previously. Given the low PPV of the combined escape and inverse pattern, these LDDST results add limited further support for such a diagnosis. The small number of dogs with these individual patterns may have influenced these results. Nevertheless, based on our study, diagnosing HAC in dogs exhibiting such results should be based on alternative criteria.

Notwithstanding the limitations of the different suppressive patterns in diagnosing HAC, demonstrating cortisol suppression during a LDDST provides additional information about the etiology of the disease in dogs with HAC.^{10,14} Approximately 60% of dogs with HAC can be determined to have PDH with the LDDST based on the identification of suppression of cortisol secretion during the test.¹⁴ A similar proportion (48%) of suppressive patterns was identified in dogs with HAC in our study. In all of these cases, PDH was confirmed by another method including ultrasonography. This finding reinforces the value of performing a LDDST in dogs in which HAC is suspected.

It has been suggested that the origin of HAC cannot be determined based on the LDDST in the remaining approximately 40% of cases with a lack of suppression pattern.¹⁴ Adrenocorticotrophic hormone secretion triggers cortisol production in healthy dogs and dogs with PDH. However, ACTH secretion is suppressed in dogs with functional AT²⁵ as a consequence of autonomous cortisol secretion. Marked increases in cortisol secretion during the LDDST suggests ACTH stimulation and, therefore, may occur in dogs with PDH but not in dogs with functional AT. Increasing cortisol concentrations during a LDDST were identified in approximately 10% of the dogs with HAC included in our study. Although the number of identified cases with this "increasing pattern" was too small for any statistical analysis to be meaningful, it was only seen in dogs with PDH and not in those with functional AT. Additional studies including a larger number of dogs with such a pattern are required to clarify if increasing cortisol concentrations can be used to identify a larger proportion of dogs with PDH using the LDDST.

The prevalence of individual clinical signs in dogs with HAC in our study was similar to that reported in earlier studies,^{3,26,27} most commonly including polyuria, polydipsia, lethargy, abdominal enlargement, excessive panting, polyphagia, and a variety of dermatological abnormalities. It has been suggested that the prevalence of various clinical signs in dogs with HAC is lower today, compared to previously.¹ Indeed, 1 report from a primary-care hospital would support such a contention.¹⁵ All of the dogs in our study had been referred for investigation and it is possible that the more severely affected and complex cases are those more likely to be referred. It could also be argued that the prevalence of clinical signs has not changed substantially, as

reflected by our findings. The single report describing a decrease in prevalence was specifically evaluating survival of untreated dogs and may have been biased toward mildly affected cases.¹⁵ A recent study evaluating dogs diagnosed with HAC in first-opinion practice reported a similar prevalence of clinical signs to that observed in our study.²⁸ However, prevalence alone was evaluated in our study, and severity of clinical signs was not assessed. Dogs today may be less severely affected, but this hypothesis would require further study.

The frequency of various clinicopathological abnormalities in dogs with HAC in our study also is similar to that reported previously,^{26,27} including increased liver enzyme activities, hypercholesterolemia, and a variety of hematological changes reflecting a stress response and thrombocytosis. Notably, in accord with previous reports,^{26,27,29} our study lends further support to the fact that dogs with HAC rarely present with a urine specific gravity > 1.030, despite retaining ability to concentrate urine if dehydrated.²⁵ By contrast, approximately one-third of dogs with NAI had urine specific gravity > 1.030. Thus, demonstration of concentrated urine may provide an inexpensive method of increasing the likelihood of NAI compared to HAC, particularly in the absence of glucosuria. In our study, additional clinicopathological abnormalities including lower lymphocyte and eosinophil counts, higher neutrophil and thrombocyte counts, lower serum creatinine concentration, higher liver enzyme and lipase activities, higher serum phosphorus and cholesterol concentrations, and proteinuria identified on urine dipstick analysis were more frequent in dogs with HAC, confirming their value in supporting such a diagnosis. Lipase activity measured by the 1,2-o-dilauryl-rac-glycero-3-glutaric acid-(6'-methylresorufin) ester assay (DGGR) was increased in approximately half of the dogs with HAC and was the next most common abnormality after increased liver enzyme activities and hypercholesterolemia. To our knowledge, this finding has not previously been reported in dogs with HAC. However, concentrations of canine pancreas-specific lipase by quantitative ELISA test (Spec cPL test, Idexx Laboratories) are increased in dogs with HAC without clinical evidence of pancreatitis compared to healthy dogs.³⁰ A good agreement and strong correlation has been shown between lipase activity measured by the DGGR assay and the Spec cPL test in several studies,^{31,32} and thus finding increased lipase activity in dogs with HAC in our study was not unexpected. Whether HAC is a predisposing factor for pancreatitis remains unresolved. Although a retrospective study suggested HAC to be a risk factor for pancreatitis,³³ several long-term studies evaluating dogs with HAC did not identify overrepresentation of pancreatitis.³⁴⁻³⁷ Lipase activity measured by the DGGR assay is not expensive and frequently included in basic biochemical panels from various laboratories. Based on our results, increased lipase activity as measured by the DGGR assay should be included in the constellation of clinicopathological abnormalities supporting a clinical suspicion of HAC.¹

Although excessive cortisol stimulation is the expected response to ACTH in HAC, it is well known that such a response does not always occur, as shown in our study.³⁻⁸ Other stimulatory patterns may provide additional information. Only 2 of 8 dogs with functional AT had a positive ACTH stimulation test result, which confirms the poor diagnostic performance of this test in this group of dogs.^{3,6} Nevertheless, 3

dogs displayed minimal cortisol stimulation which is highly suggestive of a functional AT in dogs suspicious of HAC.³⁸⁻⁴⁰ Demonstration of excess 17-hydroxyprogesterone production in these dogs proved useful diagnostically, as has been suggested previously.³⁹

Our study has additional limitations including its retrospective nature. First, the PPV (and also NPV) calculated in our study were based on a prevalence of HAC approaching 50%. Direct comparison can only be made to other studies with a similar prevalence and cannot be extrapolated to populations with a different prevalence. However, in the absence of known prevalence, it is possible from the figures presented here to calculate a likelihood ratio to impart some information on the probability of a positive result truly being positive. Second, it was difficult to demonstrate significant differences for some of the different LDDST result patterns because of the low number of cases included, and therefore wide 95% CIs were found for the escape and inverse patterns. Larger studies are required to more fully evaluate diagnostic performance of these particular patterns.

In conclusion, this retrospective study suggests that the prevalence of the different clinical and clinicopathological abnormalities in dogs with HAC today remain similar to those previously reported. Therefore, it was not surprising that the overall diagnostic test performance of LDDST remains similar to previous descriptions. Various clinicopathological abnormalities, notably lipase activity measured by the DGGR assay, can provide further supportive evidence of HAC. Interpretation of individual LDDST result patterns may provide additional diagnostic information in dogs suspicious for HAC. Primarily, a lack of suppression pattern is associated with the highest PPV for diagnosing HAC. By contrast, escape or inverse patterns are associated with a lower PPV. Not previously reported, increasing cortisol concentrations during the LDDST may support a diagnosis of PDH. Larger studies are required to confirm these findings.

CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

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