




## STANDARD ARTICLE

# Prevalence and characterization of hypoadrenocorticism in dogs with signs of chronic gastrointestinal disease: A multicenter study

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## Funding information

IDEXX Laboratories

## Abstract

**Background:** Dogs with hypoadrenocorticism (HA) frequently show signs of gastrointestinal disease (SGD). The prevalence of dogs presented for chronic SGD with HA is unknown.

**Objectives:** The aims of this study were to determine the prevalence of HA in dogs with chronic SGD and to identify clinical and laboratory variables for HA in this population.

**Animals:** One hundred fifty-one dogs with chronic SGD.

**Methods:** In this multicentered prevalence study a standardized workup was performed in prospectively enrolled dogs with SGD > 3 weeks duration. Basal serum cortisol concentration was measured in every dog with ACTH stimulation test (ACTHST) if basal serum cortisol concentration was <3 µg/dL.

**Results:** Basal serum cortisol concentration was <3 µg/dL in 80/151 (53%) dogs, <2 µg/dL in 42/151 (28%) dogs, and < 1 µg/dL in 9/151 (6%) dogs. In 6/151 dogs HA was diagnosed based on ACTHST (stimulated serum cortisol concentration < 2 µg/dL), a prevalence of 4%. There was no difference in history, physical examination, and laboratory variables between dogs with HA and those with other causes of chronic

**Abbreviations:** ACTHST, ACTH stimulation test; ALT, alanine aminotransferase; CI, confidence interval; GDH, glucocorticoid-deficient hypoadrenocorticism; GI, gastrointestinal; GIS, gastrointestinal signs; GSAVA, German Small Animal Veterinary Association; HA, hypoadrenocorticism; HPA, hypothalamus-pituitary-adrenal axis; MGDH, mineralocorticoid- and glucocorticoid deficient hypoadrenocorticism; SGD, signs of gastrointestinal disease.

This study was presented as an abstract at the 2017 American College of Veterinary Internal Medicine Forum, National Harbor, Maryland.

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SGD. In 4/6 dogs with HA, there was melena or hematochezia indicating gastrointestinal blood loss. Hyperkalemia, hyponatremia, or both was not observed in any dog.

**Conclusion and Clinical Importance:** The prevalence of HA among dogs with chronic SGD is higher than in the general population. Based on these results, testing adrenal function should be performed as a standard screening test in dogs with chronic SGD to differentiate between HA and chronic enteropathies.

#### KEYWORDS

Addison's disease, atypical hypoadrenocorticism, chronic diarrhea, cortisol

## 1 | INTRODUCTION

Hypoadrenocorticism (HA) is an uncommon endocrinopathy in dogs with prevalence ranges from 0.06 to 0.28% with 0.09% of an insured dog population in Sweden being affected.<sup>1,2</sup> There is an increased risk for HA in Portuguese Water Dogs, Standard Poodles, Bearded Collies, Cairn Terrier, and Cocker Spaniels.<sup>2</sup> There is also a genetic predisposition for HA in breeds such as the Nova Scotia Duck Tolling Retriever, Leonberger and Pomeranian, with the highest prevalence (9.73%) in Great Pyrenees dogs.<sup>3,4</sup>

It is assumed that primary HA results from a slowly progressing immune-mediated destruction and consecutive atrophy of the adrenal cortex, which is also the most common cause for Addison's disease in humans.<sup>5-7</sup> Rare other causes include trauma and infiltrative damage by neoplasia, abscess, and granulomatous inflammation.<sup>8-10</sup>

In most cases of HA gradual destruction of all 3 layers of the adrenal cortex results in an inadequate secretion of both mineralocorticoid and glucocorticoid hormones (MGDH), leading to typical electrolyte imbalances (namely hyperkalemia, hyponatremia, and often also hypochloremia) with sometimes dramatic clinical consequences.<sup>11,12</sup> However, up to 30% of dogs with HA have what has been termed atypical or glucocorticoid deficient HA (GDH) where electrolyte concentrations remain within the reference range.<sup>11</sup> The absence of typical laboratory abnormalities makes GDH more difficult to diagnose in a clinical setting.

Dogs with HA are frequently presented with vague, episodic, and nonspecific clinical signs including anorexia (89%), vomiting (72%), weight loss (42%), and diarrhea (35%).<sup>11,13</sup>

Signs of gastrointestinal disease (SGD) secondary to a lack of glucocorticoids are indistinguishable from clinical signs caused by primary GI disorders.<sup>11,14-16</sup> In the absence of electrolyte abnormalities, subtle laboratory abnormalities might lead to a suspicion of HA. These can include the lack of a stress leukogram, relative or absolute lymphocytosis, eosinophilia, prerenal azotemia, hypoalbuminemia, hypoglycemia or hypercalcemia.<sup>11,13,17</sup> However, these changes are nonspecific, have a number of possible causes and do not help to discriminate primary GI disease from HA.

Hence, diagnosis of HA, especially GDH, is dependent on adrenal gland function testing, such as ACTH-stimulation test (ACTHST).<sup>11,12,17</sup> This multicenter study was conducted in order to

evaluate the necessity of performing this relatively expensive and time-consuming test in dogs with chronic SGD. The primary aim was to assess the prevalence of HA in dogs with chronic SGD. The secondary aim was to identify clinical and laboratory variables that might help to identify or exclude HA in this group of dogs before performing ACTHST, especially in comparison to other dogs with chronic SGD that do not have HA.

## 2 | MATERIALS AND METHODS

### 2.1 | Study design and general diagnostic workup

This study was conducted prospectively by members of the German Small Animal Veterinary Association (GSAVA) Working Group for Gastroenterology at 6 different private or university referral hospitals. Ethical approval was obtained according to German animal welfare laws (approval no.: 37-24-11-2014). Each owner was informed about the purpose of the study and signed a consent form stating that blood samples were used for scientific research projects.

Between November 2014 and December 2015, dogs older than 6 months of age presenting with chronic (>3 weeks) SGD such as vomiting, regurgitation, diarrhea, weight loss, or anorexia were offered enrolment. Dogs treated with steroids were only included if (1) short-acting corticosteroids (<12 hours; eg, hydrocortisone) were given for not more than 1 week and had been discontinued for at least 1 week before enrolment; (2) intermediate-acting corticosteroids (12-36 hours; eg, prednisolone) were given for not more than 1 week and had been discontinued for at least 4 weeks before enrolment; and (3) long-acting corticosteroids (>36 hours; eg, dexamethasone) had been discontinued for at least 3 months before enrolment. These criteria were selected on the basis of a consensus between 3 endocrinologists and the authors. There are no published guidelines about the delay required for adequate adrenal function recovery after administration of different corticosteroids, and the best timing for performing an ACTH stimulation.

Clinical signs were quantified using the canine chronic enteropathy clinical activity index (CCEAI).<sup>18</sup> Diagnostic workup consisted of a complete blood count, routine biochemistry profile including electrolytes, and determination of serum TLI, cPL, folate and cobalamin concentration in all dogs.

Clinicians were given the choice to send blood count and biochemistry to 1 of 2 laboratories (IDEXX Laboratories, Ludwigsburg, Germany and Central Laboratory Animal Research Facility, University Utrecht). At IDEXX Laboratories blood count was measured using Sysmex XT and biochemistry was measured using Beckman AU5800.<sup>19</sup>

In Utrecht blood count was measured using Siemens ADVIA 2120 and biochemistry was measured using Hitachi 912 (Roche Diagnostics GmbH, Germany).

Fecal parasitology (flotation, standard egg count) was performed in every dog if recent fecal testing results from the referring veterinary surgeon were not available. Further diagnostic workup including diagnostic imaging modalities was performed based on individual clinician's decisions and availability.

## 2.2 | Endocrine testing and cortisol measurements

Basal serum cortisol concentration was determined in all dogs. An ACTHST was performed if basal serum cortisol concentration was  $<3 \mu\text{g/dL}$  ( $<83 \text{ nmol/L}$ ) or without waiting for the result of the basal cortisol dictated by the dog's clinical signs or the owner's willingness to return for the test. A basal cortisol level of  $3 \mu\text{g/dL}$  as cutoff for adrenal testing was empirically chosen to ensure not to miss any dog with hypoadrenocorticism.<sup>20,21</sup>

For the ACTHST  $5 \mu\text{g/kg}$  synthetic ACTH (Synacthen, Alfasigma S.p.A., Milan, Italy) was administered by intravenous injection and serum cortisol concentration determined before and 1 hour after ACTH administration.<sup>22</sup> The diagnosis of HA was defined by a stimulated serum cortisol concentration  $\leq 2 \mu\text{g/dL}$ .<sup>23</sup>

Cortisol concentration was measured using a solid phase, competitive chemiluminescent enzyme immunoassay (IMMULITE 2000 Cortisol, Siemens) validated for dogs.<sup>24,25</sup>

Clinicians were given the choice to send samples for cortisol determination to 1 of 2 laboratories (IDEXX Laboratories, Ludwigsburg, Germany and Central Laboratory Animal Research Facility, University Utrecht), both using the same methodology.

## 2.3 | Statistical evaluation and presentation of the data

A sample size analysis was performed using G\*Power 3. and BIAS for Windows 11.01 g software.<sup>26</sup> For evaluation of the HA prevalence a maximum prevalence of 5% was postulated and the maximum range of the 95% confidence interval was defined as 7%. For evaluation of the laboratory findings an effective size Cohen's  $d > 1$ , a  $P$ -value  $< .05$  and a minimum power of 80% was predetermined.

Statistical analyses were conducted with IBM SPSS 23.0 (IBM Germany GmbH, Ehningen, Germany), MedCalc Statistical Software version 17.9.7 (MedCalc Software bvba, Ostend, Belgium), and BIAS for Windows 11.01(Epsilon Ed., Frankfurt, Germany). Normal distribution of data was determined by Kolmogorov-Smirnoff test. Data were

compared using Fisher's exact and Mann-Whitney  $U$  test. Statistical significance was defined as  $P < .05$ .

The prevalence and its 95% confidence interval (CI) according to Wilson were calculated. A binomial test was used to compare the prevalence of HA with the 2 reported values of 0.06 and 0.28%.<sup>1</sup>

## 3 | RESULTS

Of 151 dogs (41 dogs from the Clinic of Small Animal Medicine LMU Munich Germany, 70 dogs from Small Animal Hospital Internal Medicine, Justus Liebig University Gießen, Germany and 40 dogs from Division of Small Animal Internal Medicine, Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Netherlands) enrolled in the study, 6 dogs were diagnosed with HA (all of them GDH). The calculated prevalence was 4.0% (95% CI: 1.8%-8.4%). A binomial test indicated that the proportion of dogs with SGD was significantly higher ( $P < .001$ ) than the expected prevalence of 0.06 or 0.28% (1-sided).<sup>1,2</sup>

### 3.1 | Signalment and history

#### 3.1.1 | Dogs without HA (n = 145)

Most common breeds of dogs without HA were mixed breed dogs (n = 35), Golden Retriever (n = 8), Labrador Retriever (n = 7), Poodle (n = 7), Dachshund (n = 7), German Shepherd (n = 6), Jack Russel Terrier (n = 5), Yorkshire Terrier (n = 5), French Bulldog (n = 5), Collie (n = 3), Hovawart (n = 3), Rottweiler (n = 3) and 51 other purebred dogs of 42 different breeds. Eighty-two dogs were male, of which 41 were neutered, and 63 female, of which 34 were spayed. Median age was 4.0 years (range: 1 year to 15 years) and median body weight was 17.7 kg (range: 1.8-71.7 kg).

Median duration of SGD was 5 months (range: 1-96 months). Most frequent presenting complaints were abnormal fecal or stool consistency (106/141; 75%), increased defecation frequency (85/135; 63%), mucoid feces (49/140; 35%), tenesmus (32/140; 23%), hematochezia (35/140; 32%), and melena (9/141; 6%). Fifty-two percent of dogs had a history of chronic vomiting (17% 1x/week; 12% 2-3x/week; 23% >3x/week). Median CCECAI was 5 (range: 1-20), which reflects mild disease activity.

#### 3.1.2 | Dogs with HA (n = 6)

Dogs with HA consisted of 3 mixed breed dogs, and 1 each of the following breeds: Standard Poodle, Basset hound, and German Shepherd dog. Three dogs were male, of which 1 was neutered, and 3 were spayed females. Median age was 5 years (range: 3-11 years) and median body weight was 23.9 kg (range: 8.5-31.0 kg). Median duration of SGD until presentation was 3 months (range: 1-36 months). Signs of gastrointestinal disease were abnormal fecal or stool

consistency (5/6), increased defecation frequency (5/6), mucoid feces (3/5), melena (2/6), hematochezia (4/6), and tenesmus (1/5). Four dogs had a history of chronic vomiting (1 dog 1x/week; 3 dogs 2-3x/week). The median CCECAI in the 6 dogs with HA was 6.5 (range: 3-10), reflecting a moderate disease activity.

No statistical difference concerning breed ( $P = .12$ ), age ( $P = .31$ ), sex ( $P = .75$ ), body weight ( $P = .68$ ), and severity of clinical signs ( $P = .67$ ) between dogs with and without HA was detected.

### 3.2 | Laboratory findings

Laboratory variables of dogs with and without HA are shown in Tables 1–3. There was no significant difference in any laboratory variable between groups. In dogs without HA 6 dogs had hyponatremia, 2 dogs had hyperkalemia but none of those dogs had a combination of hyponatremia and hyperkalemia. None of the 6 dogs with HA had hyperkalemia or hyponatremia, but 2 dogs with signs of

Variable	Dogs without HA		Dogs with HA		P value
	Median	Range	Median	Range	
Hematocrit (l/l)	0.50 (n = 143)	0.34-0.66	0.46 (n = 6)	0.35-0.62	.20
MCV (f/L)	71 (n = 141)	57-88	68 (n = 6)	66-77	.53
Leukocytes ( $\times 10^9/L$ )	9.3 (n = 141)	4.6-63.5	13.0 (n = 6)	5.5-26.5	.18
Monocytes ( $\times 10^9/L$ )	0.41 (n = 138)	0.14-1.48	0.53 (n = 6)	0.10-1.80	.33
Lymphocytes ( $\times 10^9/L$ )	1.71 (n = 139)	0.28-25.40	2.57 (n = 6)	0.99-5.97	.12
Neutrophils ( $\times 10^9/L$ )	6.37 (n = 137)	2.46-44.27	6.77 (n = 6)	3.86-22.30	.73
Eosinophils ( $\times 10^9/L$ )	0.46 (n = 135)	0.03-1.69	0.40 (n = 6)	0.03-1.40	.86
Thrombocytes ( $\times 10^9/L$ )	252 (n = 139)	77-875	224 (n = 6)	115-421	.54

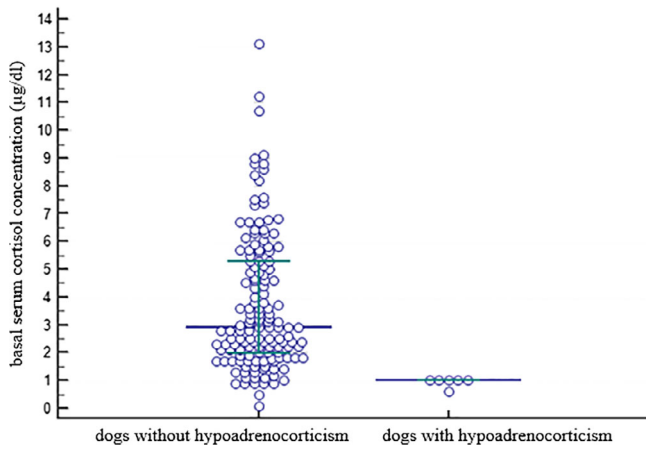
**TABLE 1** Hematology parameters of 151 dogs with chronic gastrointestinal signs grouped by presence or absence of hypoadrenocorticism (HA)

**TABLE 2** Serum biochemistry parameters of 151 dogs with chronic gastrointestinal signs grouped by presence or absence of hypoadrenocorticism (HA)

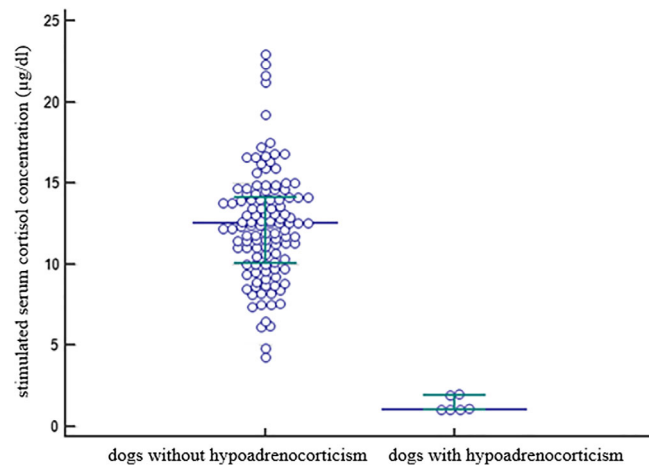
Variables	Dogs without HA		Dogs with HA		P value
	Median	Range	Median	Range	
ALT (U/L)	48 (n = 145)	9-531	91 (n = 6)	52-581	.02
AP (U/L)	41 (n = 145)	11-590	46 (n = 6)	26-678	.52
Bilirubin ( $\mu\text{mol/L}$ )	3.42 (n = 144)	0.10-6.84	3.42 (n = 6)	2.10-5.13	.70
Cholesterol (mmol/L)	5.68 (n = 142)	1.12-14.14	2.49 (n = 6)	0.90-7.59	.05
Triglyceride (mmol/L)	0.58 (n = 104)	0.24-2.75	0.36 (n = 4)	0.17-0.73	.06
Total protein (g/L)	61 (n = 145)	25-86	62 (n = 6)	53-85	.66
Albumin (g/L)	32.50 (n = 145)	7.70-39.50	35.00 (n = 6)	21.00-42.10	.38
Urea (mmol/L)	3.16 (n = 145)	1.00-18.48	4.41 (n = 6)	1.33-24.00	.42
Creatinine ( $\mu\text{mol/L}$ )	79.56 (n = 144)	26.52-282.89	77.28 (n = 6)	44.20-97.24	.76
Glucose (mmol/L)	5.11 (n = 144)	2.61-9.10	4.44 (n = 6)	3.77-6.77	.16
Folic acid (ng/mL)	10.90 (n = 131)	2.16-43.70	13.70 (n = 6)	10.00-18.20	.11
Cobalamin (pg/mL)	352 (n = 138)	106-3362	295 (n = 6)	175-363	.12

Variables	Dogs without HA		Dogs with HA		P value
	Median	Range	Median	Range	
Sodium (mmol/L)	147 (n = 144)	114-152	146 (n = 6)	142-150	.65
Potassium (mmol/L)	4.3 (n = 144)	2.6-6.1	4.5 (n = 6)	3.9-4.9	.30
Calcium (mmol/L)	2.50 (n = 144)	1.26-3.10	2.55 (n = 6)	2.30-2.80	.90
Chloride (mmol/L)	113 (n = 143)	103-121	113 (n = 6)	95-121	.90
Phosphate (mmol/L)	1.1 (n = 143)	0.5-2.2	1.3 (n = 6)	1.0-2.1	.07

**TABLE 3** Electrolyte values of 151 dogs with signs of chronic gastrointestinal disease grouped by presence or absence of hypoadrenocorticism (HA)



**FIGURE 1** Scatter plot showing basal serum cortisol concentrations in 145 dogs without hypoadrenocorticism and 6 dogs with hypoadrenocorticism. The horizontal bars represent the 25% percentile, the median and the 75% percentile



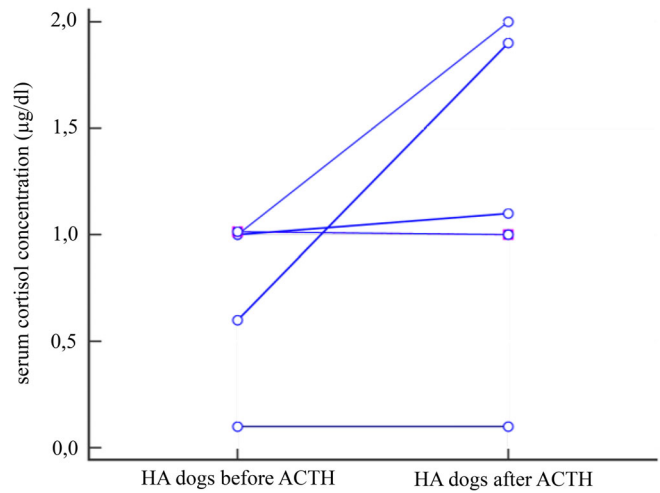
**FIGURE 2** Scatter plot showing stimulated serum cortisol concentrations in 145 dogs without hypoadrenocorticism and 6 dogs with hypoadrenocorticism. The horizontal bars represent the 25% percentile, the median and the 75% percentile

gastrointestinal bleeding had combined laboratory changes of anemia, hypoalbuminemia, and increased urea.

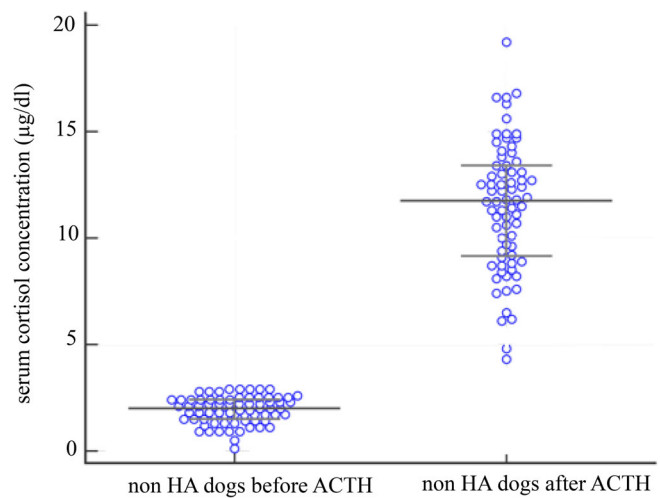
Basal serum cortisol concentration was  $\leq 3$   $\mu\text{g/dL}$  ( $\leq 83$  nmol/L) in 80/151 dogs (53%),  $\leq 2$   $\mu\text{g/dL}$  ( $\leq 55$  nmol/L) in 42/151 dogs (28%), and  $\leq 1$   $\mu\text{g/dL}$  ( $\leq 28$  nmol/L) in 9/151 dogs (6%) including all 6 dogs with HA. The basal and stimulated serum cortisol concentrations of the ACTH stimulation testing comparing dogs with HA ( $n = 6$ ) and without HA ( $n = 145$ ) are shown in Figures 1 and 2. Serum cortisol concentrations before and after the stimulation with ACTH for each individual group (dogs with and without hypoadrenocorticism) are presented in Table 4, as well as in Figure 3 and 4.

## 4 | DISCUSSION

This multicenter prospective study demonstrates a higher prevalence of HA in dogs with chronic SGD presented to several referral centers in



**FIGURE 3** Scatter plot showing serum cortisol concentrations in 6 dogs with hypoadrenocorticism before and after the stimulation with ACTH



**FIGURE 4** Scatter plot showing serum cortisol concentrations before and after the stimulation with ACTH in 74 dogs without hypoadrenocorticism and basal cortisol values  $< 3$   $\mu\text{g/dL}$

Germany and in the Netherlands (4%) in comparison to the estimated prevalence in the general canine population (between 0.06 and 0.28%).<sup>1,2</sup>

It is difficult to determine if the present study under- or overestimates the true prevalence of HA in dogs with chronic SGD. On the one hand, underestimation is possible as not all dogs suffering from HA might get referred or tested for HA. On the other hand, a referral population might be biased toward rare diseases, as it is more likely that cases that are difficult to diagnose or treat are referred, hence overestimating true prevalence in the population. In this context, it is important to stress that all dogs diagnosed with HA in the present study had GDH. The lack of typical electrolyte abnormalities decreases the suspicion for the disease and testing is less likely to take place.

The signalment of the dogs diagnosed with HA in the present study is somewhat different from previous studies, which is likely

	Dogs without HA		Dogs with HA	
	Median	Range	Median	Range
Basal cortisol µg/dL	2.9	0.1-13.1	1.0	0.1-1.0
Basal cortisol nmol/L	80.04	2.76-361.43	27.60	2.76-28.00
ACTH Stimulation (1 h) µg/dL	12.5	4.3-22.9	1.05	0.1-2.0
ACTH Stimulation (1 h) nmol/L	358.65	237.27-615.50	29.18	2.76-55.20

**TABLE 4** Serum cortisol concentrations before (basal) and after ACTH stimulation

because of the fact that classical presentations with typical electrolyte changes are missing in the present population.<sup>2,27,28</sup>

Absence of mineralocorticoid deficiency puts these dogs in a stable hemodynamic state, which can explain why they are diagnosed later compared to dogs with typical features of HA (MGDH). However, also dogs with proven mineralocorticoid deficiency can potentially maintain normal electrolyte concentrations for an extended period of time, if conditions causing hypovolemia do not occur.<sup>29</sup>

Five of six dogs with HA had signs of GI blood loss. In 4/6 dogs melena or hematochezia was noted by the owner and 1/6 dogs had anemia and hypalbuminemia most likely because of GI loss. As glucocorticoids have an important influence on epithelial integrity and vascular permeability and they can act as trophic factors for the GI mucosa, it is possible that the lack of glucocorticoids predisposes individuals to mucosal erosions and ulcers and hence hemorrhage.<sup>30,31</sup>

Neither historical information nor clinicopathological data were helpful to distinguish dogs with and without HA in the examined population. Moreover, no clinicopathological data were helpful to discriminate HA from other causes of chronic SGD.

28% of all dogs in this study had a basal serum cortisol concentration  $\leq 2$  µg/dL ( $\leq 55$  nmol/L). Dogs with chronic disease might be expected to have higher cortisol concentrations. Our results however show that chronic SGD are not generally associated with an increased stimulation of the hypothalamus-adrenal axis. This finding is consistent with other reports demonstrating that 33% of dogs with nonadrenal illness have a serum basal cortisol concentrations  $\leq 2$  µg/dL ( $\leq 55$  nmol/L).<sup>32</sup> On the other hand, a serum basal cortisol concentration  $> 2$  µg/dL ( $> 55$  nmol/L) is considered useful in excluding a diagnosis of HA.<sup>21,23</sup> In most dogs with HA serum basal cortisol concentrations are  $< 1$  µg/dL, but few dogs can have a value between 1 and 2 µg/dL. Therefore, further adrenal function testing (eg, ACTHST, cortisol: endogenous ACTH ratio) is recommended in every dog with a value  $\leq 2$  µg/dL, which according to our results might be necessary in about 1/3 of dogs with chronic SGD to definitively diagnose or rule out HA.

The authors believe all 6 dogs with inappropriate response to ACTH have true "atypical" HA. Laboratory error (both assay variation or failure as well as a clerical error) cannot be fully excluded, but ACTH stimulation tests were performed as per standard operating procedure and with due diligence. By having stringent inclusion and exclusion criteria, every effort was made to eliminate dogs from the study that might have any residual glucocorticoid effects and hence persistent suppression of the HPA from any previous treatments. However, as currently no guidelines exist regarding the required time span until ACTH testing can be performed after a dog has been

treated with different steroid formulations, a possible influence of prior steroid administration cannot be fully excluded.

This study was not set up to differentiate primary HA from secondary HA, hence endogenous ACTH was not measured. Although secondary HA is rare,<sup>33</sup> it needs to be considered in dogs with HA without electrolyte abnormalities. Determination of endogenous ACTH would also have been advantageous to rule out prior administration of glucocorticoids, which would likely result in an ACTH-stimulation mimicking hypoadrenocorticism. However, as a thorough drug history was taken for each dog it seems unlikely that the dogs identified to have no stimulation of cortisol production after ACTH administration were misdiagnosed.

Equally, aldosterone concentrations were not measured that would be necessary to document presence or absence of mineralocorticoid deficiency. Decreased aldosterone concentration could be present even in the absence of electrolyte abnormalities in some dogs with HA.<sup>29</sup>

In conclusion, the prevalence of HA among dogs with chronic SGD seen in Germany and the Netherlands is significantly higher compared to the reported prevalence in the general dog population.<sup>1,2</sup> Because approximately 1 in 25 dogs with chronic SGD might have HA, ruling out HA should be an important additional step in the standard approach of dogs with chronic SGD. Episodes of GI bleeding should specifically raise the suspicion; however, no differentiation between HA and other causes leading to chronic SGD is possible based on history, physical examination, or routine blood work. Determination of basal serum cortisol concentration should be performed as a standard screening test for HA, followed by further endocrine testing if baseline cortisol concentration is  $\leq 2$  µg/dL.

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#### CONFLICT OF INTEREST DECLARATION

Some of the hematology, serum biochemistry profiles, and serum cortisol measurements were performed free of charge by IDEXX Laboratories, Ludwigsburg, Germany.

#### 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**

Approval was obtained according to German animal welfare laws (approval no.: November 24, 2014).

**HUMAN ETHICS APPROVAL DECLARATION**

Authors declare human ethics approval was not needed for this study.

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