Received: 4 October 2021 | Accepted: 6 January 2022

DOI: 10.1111/jvim.16358

STANDARD ARTICLE

Journal of Veterinary Internal Medicine Open Access

ACVIM American College of Veterinary Internal Medicine

Urinary cortisol-creatinine ratio in dogs with hypoadrenocorticism

Francesca Del Baldo[1] | Magda Gerou Ferriani[2] | Walter Bertazzolo[3] | Matteo Luciani[3] | Antonio Maria Tardo[1] | Federico Fracassi[1]

[1]Department of Veterinary Medical Science, University of Bologna, Bologna, Italy

[2]Fitzpatrick Referrals in Soft Tissue and Oncology, Guildford, UK

[3]Laboratorio di Analisi Veterinarie MYLAV s.r.l., Milan, Italy

Correspondence
Federico Fracassi, Department of Veterinary Medical Science, Faculty of Veterinary Medicine, University of Bologna, via Tolara di Sopra, 50, 40064 Ozzano dell'Emilia, Bologna, Italy.
Email: federico.fracassi@unibo.it

Funding information
This study was partially funded by a grant provided by Laboratorio di Analisi Veterinarie MYLAV.

Abstract

Background: Basal serum cortisol (BSC) ≥2 μg/dL (>55 nmol/L) has high sensitivity but low specificity for hypoadrenocorticism (HA).

Objective: To determine whether the urinary corticoid:creatinine ratio (UCCR) can be used to differentiate dogs with HA from healthy dogs and those with diseases mimicking HA (DMHA).

Animals: Nineteen healthy dogs, 18 dogs with DMHA, and 10 dogs with HA.

Methods: Retrospective study. The UCCR was determined on urine samples from healthy dogs, dogs with DMHA, and dogs with HA. The diagnostic performance of the UCCR was assessed based on receiver operating characteristics (ROC) curves, calculating the area under the ROC curve.

Results: The UCCR was significantly lower in dogs with HA (0.65×10^{-6}; range, $0.33\text{-}1.22 \times 10^{-6}$) as compared to healthy dogs (3.38×10^{-6}; range, $1.11\text{-}17.32 \times 10^{-6}$) and those with DMHA ($10.28 \times 10^{-6}$; range, $2.46\text{-}78.65 \times 10^{-6}$) ($P < .0001$). There was no overlap between dogs with HA and dogs with DMHA. In contrast, 1 healthy dog had a UCCR value in the range of dogs with HA. The area under the ROC curve was 0.99. A UCCR cut-off value of <1.4 yielded 100% sensitivity and 97.3% specificity in diagnosing HA.

Conclusions and Clinical Importance: The UCCR seems to be a valuable and reliable screening test for HA in dogs. The greatest advantage of this test is the need for only a single urine sample.

KEYWORDS
Addison's disease, adrenal insufficiency, canine, cortisol

1 | INTRODUCTION

Hypoadrenocorticism (HA) is the umbrella term for a range of naturally occurring or iatrogenic disorders, which cause a reduced function of the adrenal cortex and result in a state of glucocorticoid deficiency, mineralocorticoid deficiency, or both.[1] In dogs, the

Abbreviations: ACTHST, ACTH stimulation test; AUC, area under the curve; DMHA, diseases mimicking hypoadrenocorticism; HA, hypoadrenocorticism; ROC, receiving operating characteristic curve; SBC, serum basal cortisol; UCCR, urinary cortisol:creatinine ratio.

majority of cases of naturally occurring HA result from primary adrenal gland failure, which is thought to be a result of the immune-mediated destruction of the adrenal cortices.[2,3]

Dogs with HA are frequently presented with vague, episodic, and nonspecific clinical signs, including anorexia, vomiting, weight loss, and diarrhea.[2-5] The most common biochemical abnormalities include azotemia and electrolyte abnormalities, such as hyponatremia, hyperkalemia, and a low-sodium-to-potassium ratio. However, up to 30% of dogs with HA have what has been defined eunatremic eukalemic HA, where electrolyte concentrations remain within the reference range.[4,6-9] The absence of typical electrolyte abnormalities makes eunatremic eukalemic HA more difficult to suspect and diagnose in a clinical setting. On the other hand, signs of gastrointestinal disease secondary to a lack of glucocorticoids are indistinguishable from clinical signs caused by primary gastrointestinal disorders.[3,10-12]

A definitive diagnosis of HA requires an ACTH stimulation test (ACTHST).[2,3] However, the high cost and limited availability of synthetic ACTH in some countries, coupled with the requirement for repeated venipuncture, are some limitations of this test. Cortisol-to-ACTH ratio also revealed a valuable tool for the diagnosis of primary HA with the greatest advantage of a single blood sample needed.[9,13,14] However, the main limitation of measurement of plasma ACTH in practice is the instability of the hormone. To avoid degradation, blood must be collected in precooled Ethylene Diamine Tetra Acetic Acid plastic tubes, processed immediately, chilled, and frozen until analysis. This procedure is time-consuming and cost-intensive.

For this reason, basal serum cortisol (BSC) concentration, an easier and cheaper screening diagnostic test, is routinely used in dogs with suspicion of HA. Using a cut-off value of ≥2 μg/dL (>55 nmol/L), the negative predictive value is reported to be between 99.8 and 100%.[15-17] However, the specificity of the test varies from 20% to 78.2%.[14-16] Therefore, due to the low specificity of the test, an ACTHST must be performed in dogs with BSC ≤2 μg/dL (≤ 55 nmol/L) to exclude HA. Since up to 33% of dogs with signs of chronic gastrointestinal disease, but without HA, which are those most commonly screened for HA in clinical practice, have an BSC <2 μg/dL (<55 nmol/L),[17,18] this means that the ACTHST must often be carried out to exclude HA, with a consequent increase in the diagnostic costs and time for the client.

The urinary corticoid:creatinine ratio (UCCR) provides an integrated measurement of corticoid production over a given interval, thereby overcoming the problem of fluctuations in plasma concentrations.[19] The greatest advantage is the need for only a single urine sample. Moreover, it is easy to carry out and relatively economical. The UCCR is currently routinely used as a screening test for dogs with spontaneous hypercortisolism,[20] and few studies have investigated its performance in monitoring dogs with hypercortisolism on a trilostane or mitotane regimen.[21-26] However, the use of the UCCR has not been evaluated in diagnosing spontaneous HA.

The aim of this study was to determine whether the UCCR could be used to differentiate dogs with HA from normal dogs and those with diseases mimicking HA (DMHA). Our hypothesis was that the UCCR would prove to have a diagnostic value in differentiating dogs with HA from dogs with DMHA.

2 | MATERIALS AND METHODS

2.1 | Animals and study design

Urine samples stored at −20°C from privately owned dogs were retrospectively selected from the University of Bologna Veterinary Teaching Hospital digital database. The urine samples had been collected from June 2019 to February 2021 from dogs with HA or DMHA at the time of diagnosis, and at routine check-ups from the healthy dogs. As per Directive 2010/63/EU of the European Parliament and of the Council of September 22, 2010, regarding the protection of animals used for scientific purposes, the Italian legislature (D. Lgs. n. 26/2014) does not require approval from ethical committees for the use of stored samples in retrospective studies.

Dogs were included in the HA group if the post-ACTH serum cortisol was ≤2 μg/dL (≤55 nmol/L), and a clinical diagnosis of naturally occurring HA was made. Dogs were excluded from the study if a glucocorticoid medication had been administered within 90 days before testing. Other dogs for which HA was suspected on the basis of clinical signs (vomiting, diarrhea, weakness, lethargy) but was subsequently excluded based on the BSC >2 μg/dL (>55 nmol/L) or ACTHST results (post-ACTH serum cortisol >5 μg/dL [>138 nmol/L])[27] were included in the DMHA group. Dogs were defined as healthy if no abnormal clinical signs were reported and if hematology, serum biochemistry, and urinalysis results were within the reference intervals.

2.2 | Sample collection and endocrine tests

For the ACTHST, blood samples were taken before and 60 minutes after the IV injection of 5 μg/kg synthetic ACTH (Synacthen, Alfasigma S.P.A., Bologna, Italy). Blood samples for the determination of cortisol were collected in serum separating tubes. Coagulated blood samples were centrifuged for 10 minutes at 3000*g*; the serum was immediately transferred to plastic tubes, stored at 4°C and analyzed the same day, or stored at −20°C and thawed immediately before analysis.

For the UCCR determination, stored urine samples were thawed at room temperature and immediately analyzed to measure creatinine and cortisol urine concentration. The urine samples were collected by free-catch (at home or in the hospital) or by US-guided cystocentesis. The UCCR was measured on the same day for all 3 groups of dogs (healthy, HA, DMHA).

3 | ANALYTICAL PROCEDURES

Serum cortisol and urine cortisol concentrations were measured with a chemiluminescent enzyme immunoassay using the antibody poll before kit lot 55 026 (Immulite 2000, Siemens Healthcare), which had been validated for dogs and is widely used in laboratories throughout the world.[28] Urine creatinine concentrations were measured using an automatic analyzer (AU480, Beckman Coulter/Olympus, Brea,

California). The UCCR was calculated from creatine and cortisol values as previously described.[29]

3.1 | Statistical analysis

Statistical analysis was carried out using commercial statistical software packages (GraphPad Prism 7, San Diego, California). Data were presented as median and range and analyzed by nonparametric tests. Differences between groups for categorical and numerical variables were analyzed using the Fisher's exact test and the Kruskal-Wallis test, respectively. The Kruskal-Wallis test followed by Dunn's post-test was carried out to compare the UCCR from dogs with HA, dogs with DMHA and healthy dogs. A receiving operating characteristic (ROC) curve was used to determine the area under the curve (AUC) and select the optimum UCCR cut-off values to diagnose or exclude HA. The ROC curve analysis was carried out by combining healthy and DMHA dogs versus HA dogs. A 95% confidence interval was calculated for the ROC curve. The level of significance was set at $P < .05$.

4 | RESULTS

4.1 | Animals

Ten dogs with HA were included. Their ages ranged from 40 to 92 months (median, 60.5 months) and their bodyweights from 3.7 to 39.6 kg (median, 13.2 kg). There were 4 males (3 castrated) and 6 females (5 spayed). The HA group consisted of 6 purebred dogs (2 Jack Russell Terriers, 1 English Setter, 1 Cocker Spaniel, 1 Rottweiler, 1 Miniature Pinscher) and 4 mixed breed dogs. All the dogs were diagnosed with primary HA. Only 1 dog had primary eunatremic eukalemic HA.

Eighteen dogs with DMHA were included. Their ages ranged from 8 to 147 months (median, 48 months) and their bodyweights from 5 to 53.5 kg (median, 24.1 kg). There were 12 males (2 castrated) and 6 females (3 spayed). This group consisted of 13 purebred dogs (2 Golden Retrievers, 1 Labrador Retriever, 2 Jack Russell Terriers, 2 Poodles, 1 Dog de Bordeaux, 1 Bernese Mountain dog, 1 French Bulldog, 1 Cavalier King Charles Spaniel, 1 Great Dane, and 1 Vizsla) and 5 mixed breed dogs. The final diagnoses were chronic gastroenteritis (12), acute gastroenteritis (4), pancreatitis (1), and adrenal neoplasia (1).

Nineteen healthy dogs were included. Their ages ranged from 13 to 98 months (median, 61 months) and their bodyweights from 6.5 to 32.0 kg (median, 23 kg). There were 7 males (4 castrated) and 12 females (8 spayed). This group consisted of 10 mixed breed dogs and 9 purebred dogs (2 Border Collie, 1 Boxer, 1 Cavalier King Charles Spaniel, 1 Lagotto Romganolo, 1 Jack Russell Terrier, 1 Spanish Greyhound, 1 Labrador Retriever, and 1 Cane Corso).

There were no significant differences between groups for age, sex, and bodyweight.

Twelve (66%) out of the 18 dogs with DMHA had BSC ≤2 μg/dL (<55 nmol/L). In these dogs, HA was excluded on the basis of post-ACTH serum cortisol >5 μg/dL (>138 nmol/L). The remaining 6 dogs had BSC > 2 μg/dL (>55 nmol/L); therefore, no additional tests were needed.

4.2 | UCCR

The median UCCR was 0.65×10^{-6} ($0.33\text{-}1.22 \times 10^{-6}$), 10.28×10^{-6} ($2.46\text{-}78.65 \times 10^{-6}$), and 3.38×10^{-6} ($1.11\text{-}17.32 \times 10^{-6}$) in dogs with HA, dogs with DMHA and healthy dogs, respectively. The median UCCR was significantly lower ($P < .0001$) in the dogs with HA as compared to the dogs with DMHA and the healthy dogs (Figure 1). There was no overlap between dogs with HA and dogs with DMHA. In contrast, 1 healthy dog had a UCCR value in the range of dogs with HA (Figure 1). The UCCR was significantly higher in dogs with DMHA as compared to healthy dogs ($P = .01$) (Figure 1). The median UCCR in dogs with DMHA and BSC ≤2 μg/dL (≤55 nmol/L) was 8.7×10^{-6} ($2.46\text{-}78.56 \times 10^{-6}$). The area under the ROC curve was 0.99 (95% CI: 0.98-1.00). A cut-off value of UCCR <1.4 revealed 100% sensitivity (95% CI: 69.1-100) and 97.3% specificity (95% CI: 85.8-99.9) in diagnosing HA.

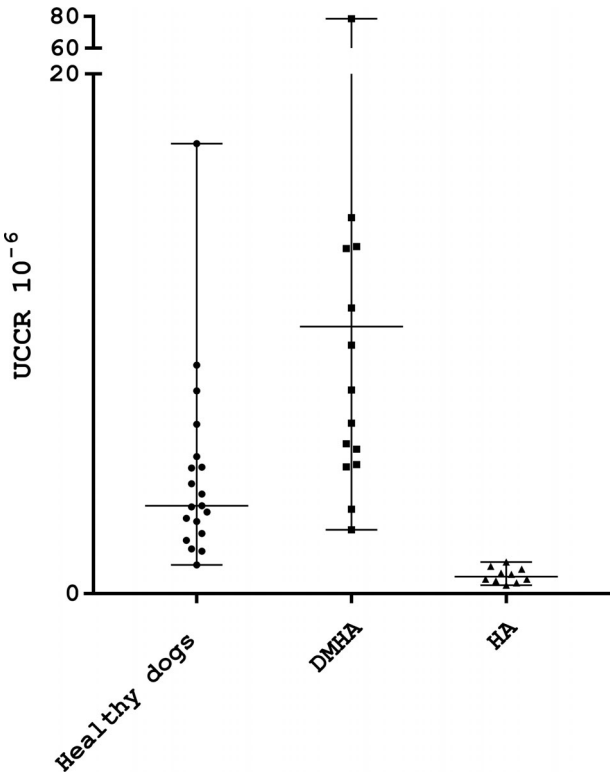

FIGURE 1 Scatter scale plot comparing urinary corticoid:creatinine ratio (UCCR) of dogs with hypoadrenocorticism (HA, n = 10), dogs with disease mimicking hypoadrenocorticism (DMHA, n = 18) and healthy dogs (healthy, n = 19). The horizontal bars represent the median, the maximum, and the minimum value of each group

5 | DISCUSSION

The results of this study show that the dogs with HA had a significantly lower UCCR than the healthy dogs and dogs with DMHA. A UCCR value >1.4×10^{-6} could be useful in excluding HA in dogs since the sensitivity of the test using this cut-off was 100%. Using the same cut-off value, the specificity of the test was 97.3%. None of the dogs with DMHA had a UCCR <1.4×10^{-6} (the lowest UCCR value detected was 2.46×10^{-6}). However, 1 healthy dog had a UCCR value <1.4×10^{-6} (UCCR = 1.11×10^{-6}).

Basal serum cortisol concentration is currently routinely used as a screening test for HA in dogs because of the evidence that BSC >2 μg/dL (>55 nmoL/L) is 100% sensitive for excluding HA.[16] However, the specificity of the test for the same cut-off is low and varies from 20% to 78.2%.[14-16] The specificity of the BSC is higher if using a cut-off <1 μg/dL (28 nmol/L) and varies from 91.5% to 98.2%.[15-17] However, the sensitivity of the test for this cut-off decreases up to 85.7%,[16] resulting in an increased number of false negatives. There are potentially serious consequences of missing a diagnosis of HA. Therefore, currently, the use of the higher cut-off >2 μg/dL (>55 nmoL/L) is advocated to exclude the disease. According to our results, the specificity of the UCCR was higher (97.3%) than the specificity of BSC. Furthermore, considering only dogs with DMHA, which are those routinely screened for HA in the clinical practice, the specificity of the test was 100%. The higher specificity of the UCCR as compared to BSC in detecting HA could be explained by the normal episodic secretion of cortisol in dogs. In this species, cortisol concentrations can become intermittently low or undetectable over a 24-hour period.[30,31] In contrast, the UCCR provides a measurement of corticoid production over a period of several hours, thereby overcoming the problem of fluctuations in plasma concentrations.[19] Although these results require confirmation by large-scale studies, the UCCR might allow a clearer differentiation between dogs with HA and dogs with DMHA. Therefore, the use of the UCCR could be an alternative screening test for HA, thus reducing costs for the owners. In addition, measuring the UCCR is less time-consuming and less invasive for the animal.

In humans, measuring urinary free cortisol levels has a low diagnostic sensitivity in detecting HA since approximately 20% of people with adrenal insufficiency have normal values.[32,33] Therefore, it is not considered a valid test for the diagnosis of HA in humans.[34] The low sensitivity of the test could be related to the severity of adrenal insufficiency with lower cortisol urinary excretion in the case of more severe adrenal insufficiency and low-normal results in patients with partial adrenal insufficiency.[35] This discrepancy between the present results and those reported in human medicine requires confirmation by large-scale studies. However, a possible explanation could be the degree of adrenal insufficiency which, in veterinary patients, could be more severe at the time of diagnosis as compared to human patients in whom the clinical signs of adrenal insufficiency are more likely to be recognized earlier compared to veterinary patients.

Up to 30% of dogs with HA have what has been called eunatremic eukalemic HA where serum electrolyte concentrations are normal at the time of diagnosis.[4,6,7,9] This subset of dogs might be more likely to undergo screening tests as opposed to a complete ACTHST given the lower index of suspicion of disease. As such, it is important to consider the diagnostic performance of UCCR in both subsets (with normal and abnormal electrolytes) of dogs. Only 1 dog included in the present study had eunatremic eukalemic HA and its UCCR was similar to the values obtained in dogs with hyponatremic HA, hyperkalemic HA, or both. However, the diagnostic utility of the UCCR in dogs with and without electrolyte abnormalities should be additionally investigated.

In this study, only dogs with spontaneous HA have been included. Dogs with iatrogenic HA receiving glucocorticoids that do not cross-react with cortisol assay, such as dexamethasone, might have a value of UCCR overlapping with those of dogs with spontaneous HA. If so, a complete and detailed clinical history would be necessary to distinguish between dogs with spontaneous and iatrogenic HA. However, to confirm this hypothesis, further studies are needed.

Measurement of cortisol-to-ACTH ratio is an alternative valuable and reliable tool for the diagnosis of primary HA in dogs.[9,13,14] Similar to the UCCR, it allows to discriminate between dogs with HA and those with DMHA with a sensitivity of 100% and a specificity of 99%.[14] However, the diagnostic utility of this test is limited in clinical practice because of the critical sampling collection and handling needed for the ACTH measurement. Moreover, the cortisol-to-ACTH ratio might be less useful compared to the UCCR in dogs with secondary HA.

The present study had several limitations. First, the small number of dogs included in each group could have markedly affected the calculated sensitivities and specificities of the UCCR to detect HA in dogs. Unfortunately, the number of dogs included in each group was limited since, few days after the analysis of the samples, there was a change in the Immulite 2000 antibody used for cortisol measurement. An initial review by the European Society of Veterinary Endocrinology—Endocrine Quality Assurance, based on >40 canine urine results, suggested that the new kit canine urine cortisol results were lower (average bias −70%) than the values obtained with the previous kit Lot (from kit Lot 550 backward).[36] Based on the above, the use of the new assay could have resulted in greater overlap between the UCCR values of dogs with HA and those with DMHA or healthy dogs. Therefore, the UCCR cut-off established in this study might need to be validated again with the new assay. Finally, due to the retrospective nature of the study, the method of urine collection was not standardized and not recorded. Veterinary care and setting could increase the overall stress level and, consequently, the UCCR in dogs.[37,38] This could have affected the results of the present study, resulting in higher UCCR values if the urine was collected in the hospital and lower if the urine was collected at home. In this regard, the collection of urine in the hospital can offer an advantage in dogs that underwent UCCR measurement as a screening test of HA. Indeed, veterinary care and setting can induce a stress response with subsequent increased serum cortisol concentration and UCCR in dogs with DMHA but not in dogs with HA, which are not able to mount a stress response because of the adrenal gland failure.

In conclusion, the determination of the UCCR seems to be a valuable and reliable screening test for HA in dogs. Using a cut-off >1.4 × 10^{-6}, differentiation between dogs with HA and those with DMHA was 100%. The most significant advantage of this test is the need for only a single urine sample.

ACKNOWLEDGMENT

This study was partially funded by a grant provided by Laboratorio di Analisi Veterinarie MYLAV. The preliminary results of this study were presented in abstract form as oral presentation at the 30th Annual Congress of the European Congress of Veterinary Internal Medicine — Companion Animals (ECVIM-CA), September 2020, online congress.

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.

HUMAN ETHICS APPROVAL DECLARATION

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

ORCID

Federico Fracassi https://orcid.org/0000-0003-3121-2199

REFERENCES

1. European Society of Veterinary Endocrinology. Project ALIVE, Term Definition "Hypoadrenocorticism"; 2020. https://www.esve.org/alive/search.aspx. Accessed March 13, 2021.
2. Peterson ME, Kintzer PP, Kass PH. Pretreatment clinical and laboratory findings in dogs with hypoadrenocorticism: 225 cases (1979–1993). *J Am Vet Med Assoc*. 1996;208:85-91.
3. Scott-Moncrieff JC. Hypoadrenocorticism. In: Feldman EC, Nelson RW, Reusch C, Scott-Moncrieff JC, eds. *Canine and Feline Endocrinology*. St. Louis, MO: Elsevier Health Sciences; 2014:485-520.
4. Thompson AL, Scott-Moncrieff JC, Anderson JD. Comparison of classic hypoadrenocorticism with glucocorticoid-deficient hypoadrenocorticism in dogs: 46 cases (1985–2005). *J Am Vet Med Assoc*. 2007;230:1190-1194.
5. Melian C, Peterson ME. Diagnosis and treatment of naturally occurring hypoadrenocorticism in 42 dogs. *J Small Anim Pract*. 1996;37:268-275.
6. Sadek D, Schaer M. Atypical Addison's disease in the dog: a retrospective survey of 14 cases. *J Am Anim Hosp Assoc*. 1996;32:159-163.
7. Lifton SJ, King LG, Zerbe CA. Glucocorticoid deficient hypoadrenocorticism in dogs: 18 cases (1986–1995). *J Am Vet Med Assoc*. 1996;209:2076-2081.
8. Hughes AM, Nelson RW, Famula TR, et al. Clinical features and heritability of hypoadrenocorticism in Nova Scotia Duck Tolling Retrievers: 25 cases (1994–2006). *J Am Vet Med Assoc*. 2007;231:407-412.
9. Lathan P, Scott-Moncrieff JC, Wills RW. Use of the cortisol- to-ACTH ratio for diagnosis of primary hypoadrenocorticism in dogs. *J Vet Intern Med*. 2014;28:1546-1550.
10. Ruckstuhl N, Hoerauf A, Tomsa K, et al. Pseudohypoadrenocorticism in two Siberian huskies with gastrointestinal parasitoses. *Schweiz Arch Tierheilkd*. 2002;144:75-81.
11. Graves TK, Schall WD, Refsal K, et al. Basal and ACTHstimulated plasma aldosterone concentrations are normal or increased in dogs with trichuriasis-associated pseudohypoadrenocorticism. *J Vet Intern Med*. 1994;8:287-289.
12. DiBartola SP, Johnson SE, Davenport DJ, et al. Clinicopathologic findings resembling hypoadrenocorticism in dogs with primary gastrointestinal disease. *J Am Vet Med Assoc*. 1985;187:60-63.
13. Javadi S, Galac S, Boer P, et al. Aldosterone-to-renin and cortisol-to-adrenocorticotropic hormone ratios in healthy dogs and dogs with primary hypoadrenocorticism. *J Vet Intern Med*. 2006;20(3):556-561.
14. Boretti FS, Meyer F, Burkhardt WA, et al. Evaluation of the cortisol-to-ACTH ratio in dogs with hypoadrenocorticism, dogs with diseases mimicking hypoadrenocorticism and in healthy dogs. *J Vet Intern Med*. 2015;29(5):1335-1341.
15. Lennon EM, Boyle TE, Hutchins RG, et al. Use of basal serum or plasma cortisol concentrations to rule out a diagnosis of hypoadrenocorticism in dogs: 123 cases (2000–2005). *J Am Vet Med Assoc*. 2007;231:413-416.
16. Bovens C, Tennant K, Reeve J, et al. Basal serum cortisol concentration as a screening test for hypoadrenocorticism in dogs. *J Vet Intern Med*. 2014;28:1541-1545.
17. Gold AJ, Langlois DK, Refsal KL. Evaluation of basal serum or plasma cortisol concentrations for the diagnosis of hypoadrenocorticism in dogs. *J Vet Intern Med*. 2016;30:1798-1805.
18. Hauck C, Schmitz SS, Burgener IA, et al. Prevalence and characterization of hypoadrenocorticism in dogs with signs of chronic gastrointestinal disease: a multicenter study. *J Vet Intern Med*. 2020;34(4):1399-1405.
19. Rijnberk A, Van Wees A, Mol JA. Assessment of two tests for the diagnosis of canine hyperadrenocorticism. *Vet Rec*. 1988;122:178-180.
20. Behrend EN, Kooistra HS, Nelson R, et al. Diagnosis of spontaneous canine hyperadrenocorticism: 2012 ACVIM consensus statement (small animal). *J Vet Intern Med*. 2013;27(6):1292-1304.
21. Angles JM, Feldman EC, Nelson RW, et al. Use of urine cortisol:creatinine ratio versus adrenocorticotropic hormone stimulation testing for monitoring mitotane treatment of pituitary-dependent hyperadrenocorticism in dogs. *J Am Vet Med Assoc*. 1997;211(8):1002-1004.
22. Guptill L, Scott-Moncrieff JC, Bottoms G, et al. Use of the urine cortisol: creatinine ratio to monitor treatment response in dogs with pituitary-dependent hyperadrenocorticism. *J Am Vet Med Assoc*. 1997;211(8):1158-1161.
23. Randolph JF, Toomey J, Center SA, et al. Use of the urine cortisol-to-creatinine ratio for monitoring dogs with pituitary-dependent hyperadrenocorticism during induction treatment with mitotane (o,p'-DDD). *Am J Vet Res*. 1998;59(3):258-261.
24. Galac S, Buijtels JJ, Kooistra HS. Urinary corticoid:creatinine ratios in dogs with pituitary-dependent hypercortisolism during trilostane treatment. *J Vet Intern Med*. 2009;23(6):1214-1219.
25. Arenas Bermejo C, Pérez Alenza D, García San José P, et al. Laboratory assessment of trilostane treatment in dogs with pituitary -dependent hyperadrenocorticism. *J Vet Intern Med*. 2020;34(4):1413-1422.
26. Golinelli S, De Marco V, Leal RO, et al. Comparison of methods to monitor different monitoring methods in dogs with hypercortisolism treated with trilostane. *J Vet Intern Med*. 2021;35(6):2616.
27. Feldman EC, Nelson RW. Hypoadrenocorticism (Addison's disease). In: Feldman EC, Nelson RW, eds. *Canine and Feline Endocrinology and Reproduction*. 3rd ed. St Louis, MO: Elsevier; 2004:394-439.
28. Singh AK, Jiang Y, White T, et al. Validation of nonradioactive chemiluminescent immunoassay methods for the analysis of thyroxine and cortisol in blood samples obtained from dogs, cats, and horses. *J Vet Diagn Invest*. 1997;9:261-268.

29. Stolp R, Rijnberk A, Meijer JC, et al. Urinary corticoids in the diagnosis of canine hyperadrenocorticism. *Res Vet Sci*. 1983;34:141-144.
30. Johnston SD, Mather EC. Canine plasma cortisol (hydrocortisone) measured by radioimmunoassay: clinical absence of diurnal variation and results of ACTH stimulation and dexamethasone suppression tests. *Am J Vet Res*. 1978;39:1766-1770.
31. Kemppainen RJ, Sartin JL. Evidence for episodic but not circadian activity in plasma concentrations of adrenocorticotrophin, cortisol and thyroxine in dogs. *J Endocrinol*. 1984;103:219-226.
32. Sonw K, Jiang NS, Kao PC, et al. Biochemical evaluation of adrenal dysfunction: the laboratory perspective. *Mayo Clin Proc*. 1992;67:1055-1065.
33. Charmandari E, Nicolaides NC, Chrousos GP. Adrenal insufficiency. *Lancet*. 2014;383(9935):2152-2167.
34. De Miguel NP, Vela ET, García NP, et al. Guidelines for the diagnosis and treatment of adrenal insufficiency in the adult. *Endocrinol Nutr*. 2014;61(1):1-35.
35. Nicolaides NC, Chrousos GP, Charmandari E. Adrenal Insufficiency. www.endotext.org. Accessed November 20, 2021
36. European Society of Veterinary Endocrinology and British Small Animal Veterinary Association Changes in canine cortisol measurement. https://www.esve.org/news/2020/20201109cortisolmeasurement_ESVE-BSAVA_Release_Nov2020.pdf. Accessed November 20, 2021
37. Van Vonderen I, Kooistra H, Rijnberk A. Influence of veterinary care on the urinary corticoid:creatinine ratio in dogs. *J Vet Intern Med*. 1998;12(6):431-435.
38. Citron LE, Weinstein NM, Littman MP, et al. Urine cortisol-creatinine and protein-creatinine ratios in urine samples from healthy dogs collected at home and in hospital. *J Vet Intern Med*. 2020;34(2):777-782.

How to cite this article: Del Baldo F, Gerou Ferriani M, Bertazzolo W, Luciani M, Tardo AM, Fracassi F. Urinary cortisol-creatinine ratio in dogs with hypoadrenocorticism. *J Vet Intern Med*. 2022;1-6. doi:10.1111/jvim.16358