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CASE REPORT

Companion or pet animals

Crooke's cell pituitary adenoma in a 7-month-old dog with Cushing's syndrome

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Abstract

A 7-month-old Boxer puppy was referred for growth retardation. Cushing's syndrome was diagnosed based on clinical signs, physical appearance, elevated urine cortisol/creatinine ratios and unsuppressed plasma cortisol after a low dose of dexamethasone. At the time, there was no evidence of pituitary or adrenal neoplasia on imaging studies. After 60 days of trilostane treatment, the clinical signs of Cushing's syndrome had disappeared. Thirty months later, the dog died of cardiac disease. Pathological examination revealed a pituitary macroadenoma with bilateral adrenocortical hyperplasia. Immunostaining for adrenocorticotropic hormone confirmed a corticotroph adenoma. High Ki-67 staining denoted the aggressiveness of the tumour. Haematoxylin–eosin, periodic acid-Schiff and cytokeratin 7 staining revealed a perinuclear hyaline ring in the neoplastic cells, compatible with a Crooke's cell adenoma. This is the first case of Crooke's cell pituitary adenoma causing Cushing's syndrome in a puppy with growth retardation.

BACKGROUND

Cushing's syndrome (CS), that is hypercortisolism, is one of the most common endocrine disorders in dogs. It usually occurs in middle-aged and elderly dogs and in the majority of cases is due to a pituitary corticotroph adenoma secreting excessive amounts of adrenocorticotropic hormone (ACTH).[1] Other forms of CS described in dogs are cortisol-secreting adrenocortical tumours, ectopic ACTH syndrome, food-dependent hypercortisolism (due to aberrant expression of glucose-dependent insulinotropic peptide receptor) and primary bilateral adrenocortical hyperplasia (due to intra-adrenocortical ACTH expression).[2–6] All of these forms of CS have been described in adult dogs. To the authors' knowledge, no case of spontaneous CS in a young dog with hitologic confirmation of pituitary tumour has been described. However, five young dogs with spontaneous CS have been described, of which only in an 8-month-old Shiba Inu dog was the diagnosis documented by hormonal testing, in a 6-month-old experimental beagle, it was suspected by histological findings of the adrenal glands, and the remaining three cases (6, 12 and 18 months of age) were mentioned in an endocrinology textbook.[7–9]

The World Health Organization has classified human pituitary tumours into adenomas, carcinomas and a rare subtype of tumours called aggressive pituitary tumours (APTs).[10] Of all human pituitary tumours, 10% occur in the pediatric population. The most common APTs in children and adolescents are giant prolactinoma, giant somatotropinoma, silent corticotroph adenoma, pituitary carcinoma and Crooke's cell adenoma.[10]

Crooke's cell adenomas, first described by Arthur Carleton Crooke in 1935 (Crooke 1935), are a rare and aggressive subtype of corticotroph adenomas in humans, with only five cases reported in the pediatric population, two of them with clinical signs of CS.[10] This distinct clinicopathological subtype quite often presents as a macroadenoma and has a relatively high recurrence rate after adenomectomy.[11] Crooke's cell are characterised by accumulation of cytokeratin filaments around the nucleus, which seems to be associated with the effects of glucocorticoid excess on the corticotrophic cells. Polledo et al. reported the histopathological features of pituitary adenomas in 136 dogs, and found Crooke-like changes in non-neoplastic corticotroph cells in four corticotroph adenomas.[12] To the authors' knowledge, Crooke's cell pituitary adenoma has not been reported in dogs to date.

This case report represents the first description of a young dog, presented because of growth retardation, in which spontaneous CS was proven. Histopathological examination revealed Crooke's cell pituitary adenoma.

CASE PRESENTATION

A 7-month-old, entire, male Boxer (bodyweight 22 kg) was referred for growth retardation (height 42 cm) compared to its littermate (height 49 cm). The dog was eating a high-quality commercial puppy food, had age-appropriate

vaccinations and deworming and was not receiving any medication. Polyuria, polydipsia and polyphagia were the signs described by the owner. Physical examination revealed overweight (body condition score 6/9), proportional growth retardation (compared to its brother from the same litter), pot-bellied appearance, thin and inelastic skin with striae and superficial pyoderma (Figure 1). Goiter or alteration of consciousness (alert sensorium) were not found.

INVESTIGATIONS

Routine laboratory examination showed increased alanine aminotransferase activity (504 U/L; reference range [RR]: <50 U/L), aspartate aminotransferase activity (138 U/L; RR: <50 U/L) and fasting total cholesterol (516 mg/dL; RR: <220 mg/dL). The urine specific gravity was low (1.015) and the urine did not contain glucose. Non-endocrine causes of growth retardation were ruled out through anamnesis, physical examination, complete blood count, routine blood biochemistry, urinalysis, coproparasitology, chest x-ray and ultrasound of the abdomen. Although hypothyroidism and growth hormone (GH) deficiency were considered as endocrine causes of growth retardation, the marked clinical picture strongly pointed to CS.

Circulating concentrations of total thyroxine (TT_4), thyrotropin (TSH), insulin-like growth factor 1 (IGF-1) and urinary and plasma cortisol concentrations were measured using a homologous solid-phase, chemiluminescent enzyme immunoassay (Immulite 1000, Siemens Healthineers, Argentina) in accordance with the manufacturer's instructions.[13,14]

Normal circulating concentrations of TT_4 (1.3 μg/dL, RR: 1–3 μg/dL) and TSH (0.20 ng/mL, RR: <0.5 ng/mL) ruled out hypothyroidism. A low serum IGF-1 concentration (24 ng/mL, brother: 432 ng/mL) pointed to GH deficiency, which could explain the growth retardation. The urine cortisol/creatinine ratio in three samples collected by the owner at home on different days was severely elevated ($>400 \times 10^{-6}$, RR: $<15 \times 10^{-6}$). Additionally, the unsuppressed plasma cortisol concentration after a low dose (0.01 mg/kg) of intravenous dexamethasone at 8 hours (1.8 μg/dL, RR: <1.2 μg/dL) ultimately confirmed the diagnosis of CS. Adrenal ultrasound and pituitary magnetic resonance imaging performed to define the origin of CS in this case did not reveal a pituitary or adrenal tumour. Although endogenous ACTH measurement could have been useful to define the pituitary origin of CS, it was not possible to perform. Because of the absence of an adrenal tumour, the dog was diagnosed with CS due to a pituitary microadenoma.

TREATMENT

Treatment for CS was initiated with the 3-betahydroxysteroid dehydrogenase enzyme inhibitor trilostane (Oncovet TL, ChemovetPharmaceutical, Argentina; at a dose of 1 mg/kg orally twice daily). On Day 14 after the start of the treatment, an ACTH stimulation test (Synacthen 0.25 mg, Novartis, Argentina) was performed 3 hours after trilostane administration using a dose of 5 μg/kg intravenous. The ACTH stimulation test revealed adequate adrenocortical reserve with a post-ACTH plasma cortisol concentration of 1.98 μg/dL.[15] At 2-month follow-up, there was complete remission of polyuria, polydipsia and pyoderma. The serum IGF-1 concentration had increased to 575 ng/mL (brother, 495 ng/mL). The circulating TT_4 and TSH concentrations were 2.2 μg/dL and 0.38 ng/mL, respectively. At 9 months of treatment, the dog showed no signs or physical appearance of CS (Figure 2), but was smaller in size than its brother. An ACTH stimulation

LEARNING POINTS/TAKE-HOME MESSAGES

- Natural Cushing's syndrome can occur in puppies.
- Excess cortisol can induce growth retardation in puppies.
- Crooke's cell pituitary adenomas are aggressive tumours in children, and exceptionally cause Cushing's syndrome.
- Crooke's cell pituitary adenoma also occurs in dogs.

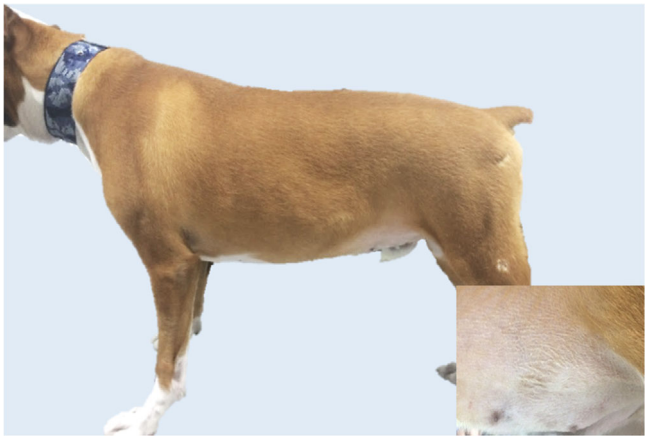
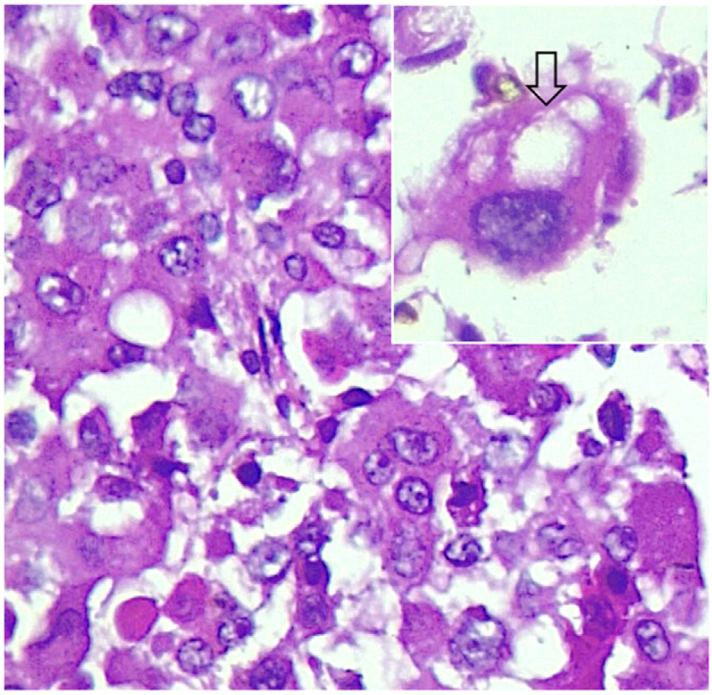


FIGURE 1 Male Boxer puppy with Cushing's syndrome and growth retardation at 7 months of age. The abdomen is pot-bellied with thin skin with striae (lower right inset).

FIGURE 2 The dog at 9 months after initiation of trilostane treatment, without clinical expression of Cushing's syndrome.

FIGURE 3 Cross-section of the brain at the level of the diencephalon (left) showing the large pituitary tumour (asterisk) and both enlarged adrenal glands with normal shape and without evidence of adrenal cortex tumour (right).

FIGURE 4 Staining of Crooke's cell pituitary adenoma (corticotroph adenoma) in a Boxer dog. The cytoplasm of neoplastic cells shows a perinuclear and hyaline ring (arrow) indicative of Crooke's hyaline changes. Haematoxylin and eosin staining.



test revealed adequate adrenocortical reserve and circulating TT_4 and TSH concentrations were within reference ranges.

OUTCOME AND FOLLOW-UP

The dog was monitored biannually, but at 30 months of age clinical signs of cortisol excess reappeared, which were maintained even with a trilostane dose (4 mg/kg twice daily). At 39 months of age, the dog died of cardiac arrhythmias secondary to dilated cardiomyopathy.

A postmortem examination was performed, which revealed a large pituitary neoplasm (1.5 cm in diameter) and bilateral enlarged adrenal glands (Figure 3). The pituitary and both adrenal glands were fixed in 10% neutral buffered formalin, and 4-μm-thick histologic sections were routinely prepared and stained with haematoxylin and eosin, periodic acid-Schiff (PAS) and reticulin stain. Immunohistochemical staining for ACTH (1/2000), cytokeratin 7 (1/50) and proliferative marker Ki-67 (1/50) were performed on the pituitary tissue sections.

The pituitary tissue section showed a PAS-positive pituitary adenoma with nuclear pleomorphism. The cells were arranged in nests with loss of the reticulin network. Haematoxylin–eosin, PAS and cytokeratin staining showed strong perinuclear ring staining in the cells constituting the adenoma, a characteristic feature of human Crooke's cell adenoma (Figures 4–6). Immunostaining for ACTH was peri-cytoplasmic or marginal, confirming corticotroph adenoma (Figure 7). A high percentage of neoplastic cells was Ki-67-positive (10%), denoting the aggressiveness of the adenoma (Figure 8). Hyperplasia of the fascicular and reticular zone was found in both adrenal glands.

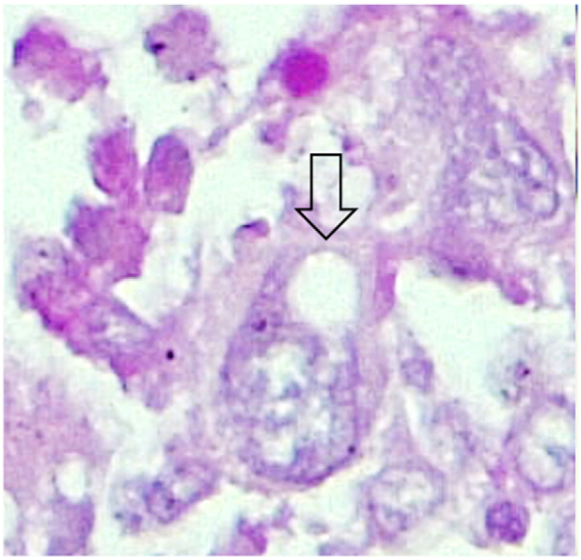


FIGURE 5 Staining of Crooke's cell pituitary adenoma (corticotroph adenoma) in a Boxer dog. The cytoplasm of neoplastic cells shows a perinuclear and hyaline ring (arrow) indicative of Crooke's hyaline changes. Periodic acid-Schiff staining.

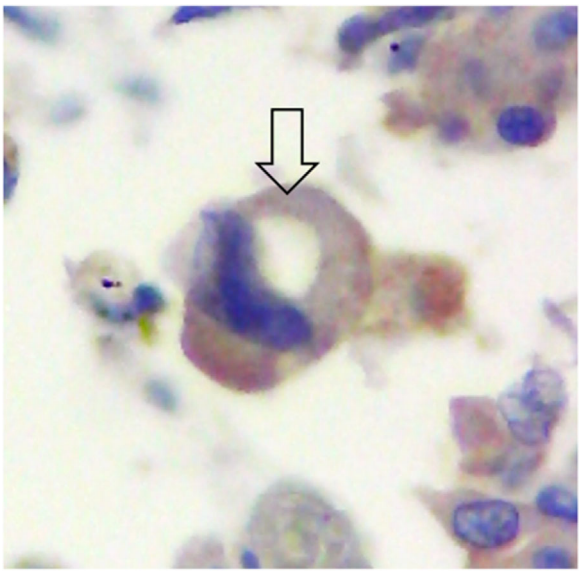


FIGURE 6 Staining of Crooke's cell pituitary adenoma (corticotroph adenoma) in a Boxer dog. The cytoplasm of neoplastic cells shows a perinuclear and hyaline ring (arrow) indicative of Crooke's hyaline changes. Cytokeratin 7 immunohistochemistry staining.

DISCUSSION

The initial complaint of this dog was growth retardation. Other clinical signs, including polyuria, polydipsia, polyphagia, skin atrophy and pot-bellied appearance, pointed to hypercortisolism. Chronic hypercortisolism can induce growth retardation by affecting GH secretion (increased somatostatin tone) and the development of chondrocytes present in the growth plates of the long bones.[16,17] The low circulating IGF-1 concentration was consistent with GH deficiency in this case, and restored during medical treatment of CS.

Other endocrine causes of growth retardation in dogs include hypothyroidism and juvenile diabetes mellitus.[18] The absence of altered mental status, goiter and disproportionate growth together with circulating thyroid hormone concentrations within the reference range made the diagnosis of hypothyroidism unlikely in this case. Diabetes mellitus was ruled out due to absence of hyperglycemia and glycosuria.

Another differential diagnosis in a puppy with growth retardation associated with a pituitary tumour and GH deficiency is craniopharyngioma. However, these tumours also cause deficiency of other pituitary hormones (panhypopituitarism), and have not been described in dogs with CS.[19]

The diagnosis of CS in this young dog was made as in adult dogs. Magnetic resonance imaging at the time of diagnosis did not reveal an enlarged pituitary, possibly due to the short time elapsed of the disease. However, the pituitary tumour found at postmortem examination, and confirmed on histopathology, defined the pituitary origin of CS in this case.

The initial response to CS treatment with trilostane was excellent, with disappearance of clinical signs and normalisation of the biochemical profile for almost 30 months. Unfortunately, in the last period of the animal's life, clinical signs associated with cortisol excess were uncontrollable with trilostane. This may be attributed to the progressive growth of the pituitary neoplasm, possibly induced by the loss of cortisol negative feedback (biochemical Nelson syndrome), or to the aggressive nature of the neoplasm.[20,21] The high Ki-67 staining and the type of tumour, that is Crooke's cell pituitary adenoma, underline the aggressive nature of the neoplasm.[22]

At postmortem examination and histology, we found bilateral adrenal enlargement (corticoadrenal hyperplasia) and a large pituitary tumour, which was confirmed by immunohistochemistry as an ACTH-positive corticotroph adenoma. However, most of the cells of this tumour showed hyaline changes in the cytoplasm compatible with Crooke's cells in humans. This hyaline change is due to the massive accumulation of perinuclear cytokeratin filaments that can be identified by immunohistochemistry (cytokeratin), and is usually seen in non-neoplastic corticotroph cells in patients with glucocorticoid excess, but may also be seen in a rare corticotroph tumour called a Crooke's cells adenoma.[8,19] These 'ring shape' cells and cytokeratin positivity were observed in this case. In the typical corticotroph adenomas, these changes are not present. Crooke's cell adenomas are unusual and aggressive pituitary adenomas with elevated Ki-67 (>3%) staining.[10,21] With a Ki-67 score of 10%, compared to a mean score of about 1.6% for canine corticotroph adenomas, the adenoma of our dog was defined as aggressive.[22]

To our knowledge, Crooke's cell adenoma causing CS has not been previously described in dogs, implying that this is the first case reported in canine medicine. Moreover, it occurred in a puppy, indicating that CS may occur in very young dogs. It also illustrates that cortisol excess at a young age may result in growth retardation in dogs.

AUTHOR CONTRIBUTIONS

Santiago Teyssandier and Elber Alberto Soler Arias were the endocrinologists in charge of the clinical care of the case and the writing of the article. Silvina Figurelli was in charge of the histopathologic diagnosis. Sara Galac and Hans Kooistra did the critical correction of the manuscript.

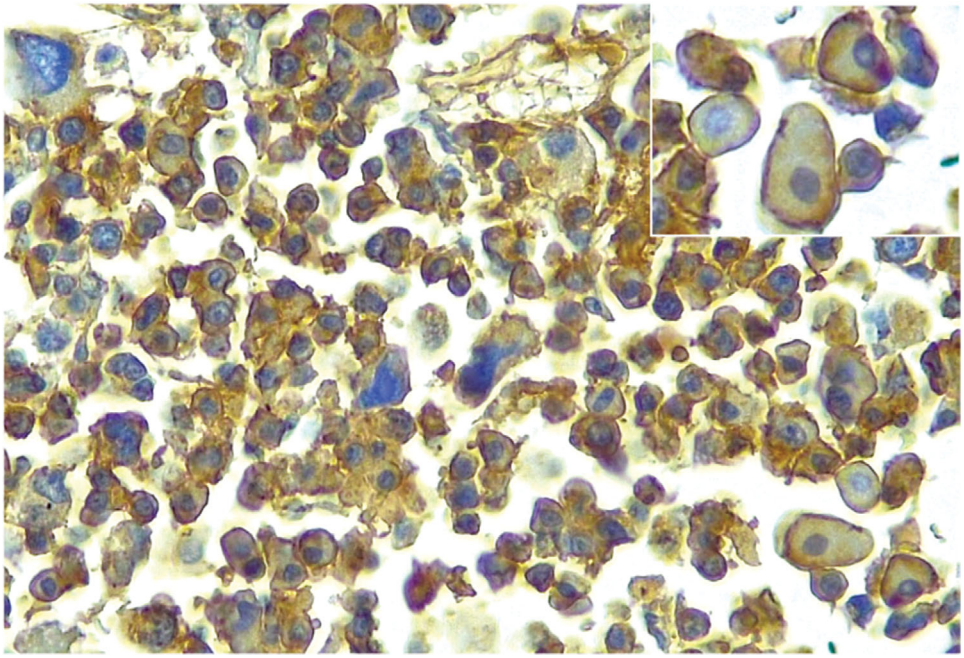


FIGURE 7 Staining of Crooke's cell pituitary adenoma (corticotroph adenoma) in a Boxer dog. Adrenocorticotropin hormone immunohistochemistry staining.

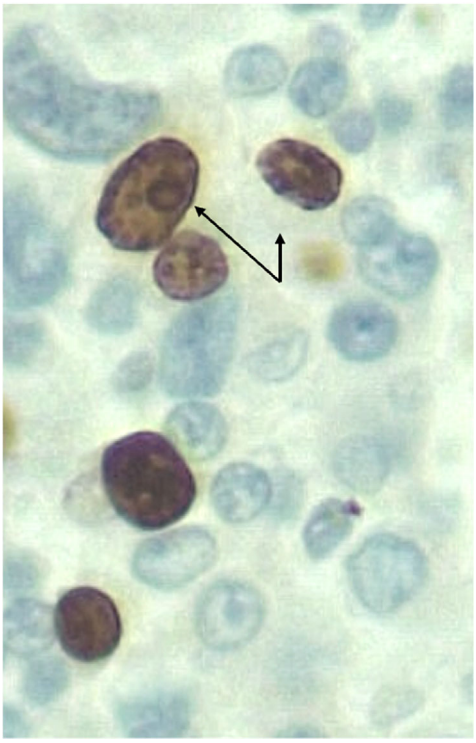


FIGURE 8 Staining of Crooke's cell pituitary adenoma (corticotroph adenoma) in a Boxer dog. Positive nuclear immunohistochemistry staining (arrow) for proliferation factor Ki-67.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest that could be perceived as prejudicing the impartiality of the research reported.

ETHICS STATEMENT

The work described in this manuscript involved the use of non-experimental animals and established internationally recognised high standards ('best practice') of veterinary clinical care for the individual. Ethical approval from a committee was therefore not specifically required for publication in Vet Record Case Reports. Additionally, written consent was obtained from owner for the publication.

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REFERENCES

1. Sanders K, Galac S, Meij BP. Pituitary tumour types in dogs and cats. Vet J. 2021;270:105623.
2. Castillo VA, Pessina PP, Garcia JD, Hall P, Gallelli MF, Cabrera Blatter MF. Ectopic ACTH syndrome in a dog with a mesenteric neuroendocrine tumour: a case report. Vet Med. 2014;59(7):352–8.
3. Churcher RK. Hepatic carcinoid, hypercortisolism and hypokalemia in a dog. Aust Vet J.1999;77(10):641–5.
4. Galac S, Kooistra HS, Voorhout G, van den Ingh TSGAM, Mol JA, van den Berg G, et al. Hyperadrenocorticism in a dog due to ectopic secretion of adrenocorticotropic hormone. Domest Anim Endocrinol. 2005;28(3):338–48.
5. Galac S, Kars VJ, Voorhout G, Mol JA, Kooistra HS. ACTH-independent hyperadrenocorticism due to food-dependent hypercortisolemia in a dog: a case report. Vet J. 2008;177(1):141–3.
6. Soler Arias EA, Castillo VA, Louiset E, Lefebvre H. Cushing's syndrome caused by intra-adrenocortical adrenocorticotropic hormone in a dog. J Vet Intern Med. 2022;36(1):264–71.
7. Nagamatsu R, Nagata M. Juvenile-onset, ACTH-dependent Cushing's syndrome in a dog. Jpn J Vet Dermatol. 2009;15:145–6.
8. Ryo DO, Yuki K, Yoshiji A, Miho M, Keigo M, Kae F, et al. Suspected spontaneous hyperadrenocorticism in a young experimental beagle dog. J Toxicol Pathol. 2021;34(3):261–7.
9. Behrend EN. Canine hyperadrenocorticism. In: Feldman EC, Nelson RW, Reusch CE, Scott-Moncrieff JCR, Beherend EN, editors. Canine and feline endocrinology. 4th ed. St Louis, MO, USA: Elsevier; 2015. p. 386–7.
10. Tatsi C, Stratakis CA. Aggressive pituitary tumors in the young and elderly. Rev Endocr Metab Disord. 2020;21:213–23.

11. Zhu D, Wang Z, Tian T, Xinyi W, Dongsheng H, Yonghong Z, et al. Prevalence and clinical characteristics of Crooke's cell adenomas in 101 patients with T-PIT-positive pituitary adenomas: case series and literature review. Front Endocrinol. 2022;13:947085.
12. Polledo L, Grinwis GCM, Graham P, Dunning M, Baiker K. Pathological findings in the pituitary glands of dogs and cats. Vet Pathol. 2018;55:880–8.
13. Singh AK, Jiang Y, White T, Spassova D. 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;9:261–8.
14. Tvarijonaviciute A, Tecles F, Carrillo JM, Rubio M, Ceron JJ. Serum insulin-like growth factor-1 measurements in dogs: performance characteristics of an automated assay and study of some sources of variation. Can J Vet Res. 2011;75(4):312–6.
15. Macfarlane L, Parkin T, Ramsey I. Pre-trilostane and 3 h post-trilostane cortisol to monitor trilostane therapy in dogs. Vet Rec. 2016;179(23):597.
16. Meij B, Mol JA, Bevers M, Rijnberk A. Alterations in anterior pituitary function of dogs with pituitary-dependent hyperadrenocorticism. J Endocrinol. 1997;154(3):505–12.
17. Minnetti M, Caiulo S, Ferrigno R, Baldini-Ferroli B, Bottaro G, Gianfrilli D, et al. Abnormal linear growth in pediatric adrenal diseases: Pathogenesis, prevalence and management. Clin Endocrinol. 2020;92:98–108.
18. Reusch CE. Disorders of growth hormone. In: Feldman EC, Nelson RW, Reusch CE, Scott-Moncrieff JCR, Beherend EN, editors. Canine and feline endocrinology. 4th ed. St Louis, MO, USA: Elsevier; 2015. p. 37–76.
19. Rosol TJ, Meuten DJ. Tumors of the endocrine glands. In: Meuten DJ, editor. Tumors in domestic animals. 5th ed. Wiley Blackwell; 2016. p. 768–82.
20. Teshima T, Hara Y, Takekoshi S, Nezu Y, Harada Y, Yogo T, et al. Trilostane-induced inhibition of cortisol secretion results in reduced negative feedback at the hypothalamic-pituitary axis. Domest Anim Endocrinol. 2009;36(1):32–44.
21. George DH, Scheithauer BW, Kovacs K, Horvath E, Young WF Jr, Lioyd RV, et al. Crooke's cell adenoma of the pituitary: an aggressive variant of corticotroph adenoma. Am J Surg Pathol. 2003;27(10):1330–6.
22. Ishino H, Hara Y, Takekoshi S, Teshima T, Teramoto A, Osamura RY, et al. Ki-67 and minichromosome maintenance-7 (MCM7) expression in canine pituitary corticotroph adenomas. Domest Anim Endocrinol. 2011;41(4):207–13.

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