

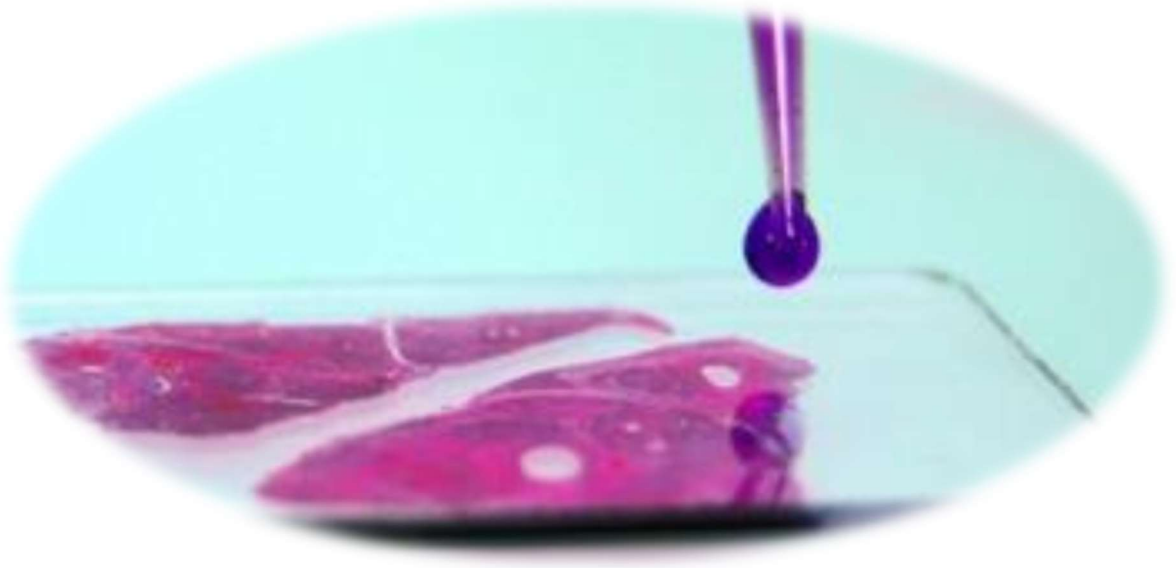
PITUITARY PATHOLOGICAL REVIEW IN 201 DOGS AND CATS

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THESIS SUBMITTED FOR MASTERS OF VETERINARY MEDICINE



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A large section of this work has been submitted to publication in Veterinary Pathology journal or has been published as Polledo et al., 2017 “Hypophysitis, panhypopituitarism and hypothalamitis in a Scottish terrier dog”.

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List of abbreviations

• ACTH	Adrenocorticotrophic hormone
• ADH	Antidiuretic hormone
• CT	Computerised tomography
• FSH	Follicle-stimulating hormone
• GH	Growth hormone
• HE	Hematoxyline-eosine
• LH	Luteinizing hormone
• MRI	Magnetic resonance imaging
• MSH	Melanocyte stimulating hormone
• PAS	Periodic acid-Schiff positive
• PRL	Prolactin
• TSH	Thyroid-stimulating hormone
• T1W	T1-weighted
• T2W	T2-weighted

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1 RESEARCH PROJECT

1.1 Brief summary and justification of the work

In this study, we aimed to investigate the prevalence of pituitary changes and the range of its lesions in a general population of 201 dogs and cats. Clinical data, macroscopic and histopathologic data were carefully evaluated and further tests like special stains and immunohistochemical tests were applied and its significance is discussed in the context of the current literature, adding to the current knowledge of pituitary changes in dogs and cats, highlights debatable aspects of the current classification and helps pathologists and veterinarians with the diagnostic process.

A wide range of abnormalities can occur within the pituitary gland which can lead to various endocrinological or neurological symptoms. With the exception of classic functional adenomas in dogs and horses, pituitary lesions are infrequently described in the veterinary literature. Given the increasing availability of advanced imaging in the diagnostic approach of both endocrine and neurological disease, a better understanding of the range of abnormalities encountered in the pituitary gland is desirable, especially with the progression in sampling or surgical treatment of the pituitary gland in dogs and cats. Approximately 10% of pituitary glands from asymptomatic humans contain abnormalities but the equivalent proportion in small animals is unknown.

Pituitary glands from 136 dogs and 65 cats collected during routine necropsies were examined to determine the prevalence of pituitary lesions and their histopathological diagnosis. Sections were prepared with haematoxylin and eosin (HE), periodic acid-Schiff (PAS), Gordon and Sweet's reticulin and immunohistochemistry against several secreting hormonal peptides. Pituitary abnormalities were identified in 36/136 (26.4%) dogs and 10/65 (15.3%) cats. Pituitary cysts were the commonest lesion in dogs and cats, occurring in 18 (13.2%) dogs and 8 (12.3%) cats. Pituitary neoplasia was detected in 14.1% (12/85) of middle/old aged dogs; 1 (1.5%) cat had pituitary nodular hyperplasia. PAS proved useful for staining secretory granules in adrenocorticotrophic hormone (ACTH)-positive adenomas and reticulin stain helped differentiate them from hyperplasia. These tumours were uniformly PAS positive with a loss of the normal reticulin network. One dog had a pituitary carcinoma with marked infiltration into the dorsal thalamus.

Other pituitary abnormalities included: secondary metastatic neoplasia (2 dogs), hypophysitis (4 dogs, 1 cat). In the majority of cases the lesion was subclinical and could be considered incidental; of those dogs and cats with pituitary lesions, only 4 dogs (2.9%) and

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no cats demonstrated clinical manifestations of their pituitary disease ante mortem. Pituitary abnormalities are common in dogs and cats and their wider clinical relevance requires further investigation.

Derived from this work and in collaboration with clinicians from Pride Veterinary Hospital, a unique case report of a dog presenting with inflammatory hypophysitis and hypothalamitis of suspected autoimmune origin with detailed clinical, magnetic resonance imaging (MRI), histology and immunohistochemistry findings which to our knowledge was reported. The case was initially misdiagnosed as a tumour by MRI; the correct diagnosis was made at post-mortem. We feel the value of this case report is in alerting clinicians that inflammatory conditions of the pituitary gland should be considered as potential differential diagnoses of suprasellar masses. Lymphocytic hypophysitis should be considered in the differential diagnoses of pituitary mass lesions, because it warrants very different management and treatment. In addition to the new information relevant to MRI interpretation, the report contains important clinical and pathological information on this previously unreported lesion. The study of the case has been thorough, including the integration of several specialities and has the potential to improve the diagnosis of future cases.

1.2 Introduction

1.2.1 Anatomy and function of the pituitary gland

The pituitary gland is an endocrine organ located intracranially in the sellar region, which in concert with the hypothalamus and nervous system regulates the metabolism, growth, development, tissue function and maintains the internal homeostasis of the body ³¹. It is composed of two morphologically and functionally distinct components: the anterior lobe (adenohypophysis), and posterior lobe (neurohypophysis or *pars nervosa*).

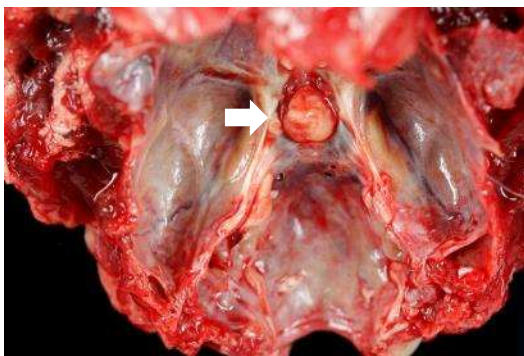


Figure 1 Base of the cranium, dog. Pituitary gland within the sellar region (white arrow).

The **adenohypophysis** (*pars distalis and intermedia*) is formed by several distinct cell populations producing different hormones with different staining characteristics. Secretory cells are classified based on their appearance in hematoxyline-eosine stains as: Acidophils, that comprise somatotrophs cells that secrete growth hormone (GH), and lactotrophs that secrete prolactin (PRL); basophils, that also stain periodic acid-Schiff positive (PAS), and include gonadotrophs that produce luteinizing hormone (LH) and follicle-stimulating hormone (FSH) and thyrotrophs that secrete thyroid-stimulating hormone (TSH), and cytoplasmic poorly-stained chromophobes, ACTH-or melanocyte stimulating hormone (MSH) secreting corticotrophs; and the posterior lobe (neurohypophysis) which consists of modified glial cells and axonal processes extending from the hypothalamus. Indeed, oxytocin and antidiuretic hormone (ADH) contained in the neurohypophysis are synthesized in the hypothalamus and stored within the axon terminals in the posterior lobe ⁵⁹.

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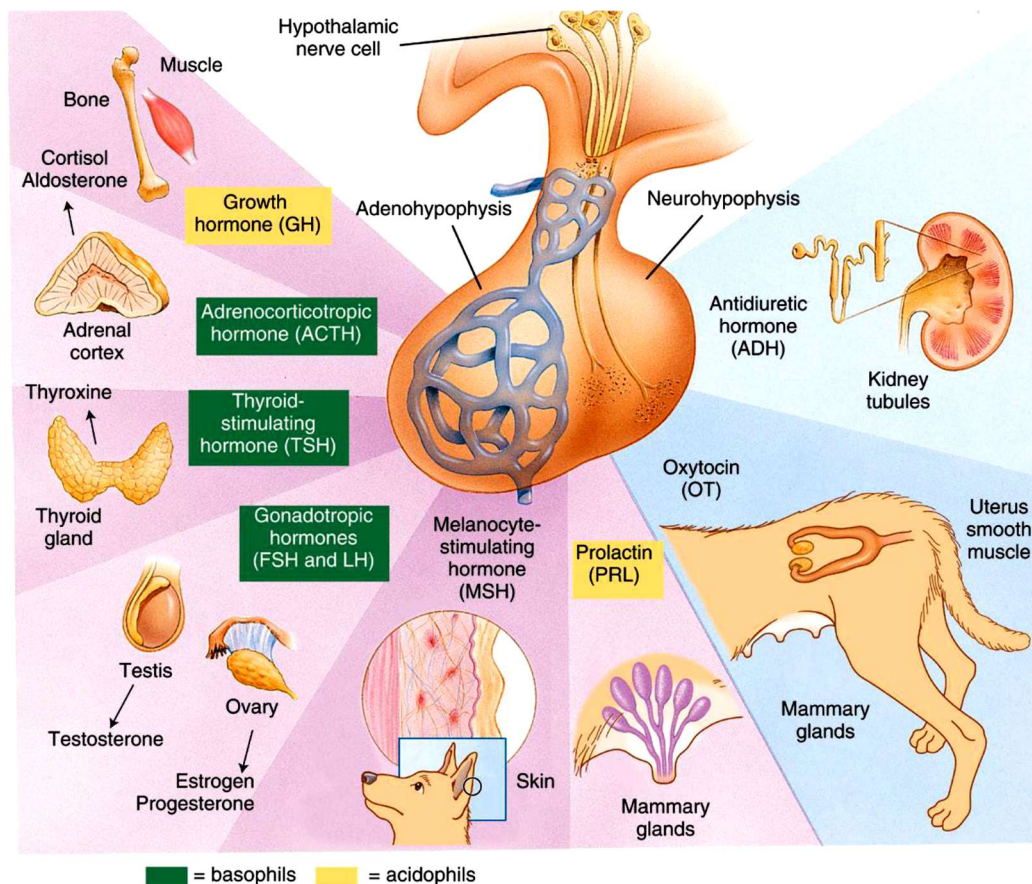


Figure 2 Anatomy of the pituitary gland and hormonal secretion. From: JF Zachary; MD McGavin. Book: Pathologic basis of veterinary disease (2016)

1.2.2 Pathology of the pituitary gland

Knowledge of pathological changes is important to make the right diagnosis, especially in a time where marked advances in neuroimaging have notably improved the identification of pituitary disease in small animals, rendering sampling or surgical treatment of the pituitary gland through transsphenoidal hypophysectomy possible albeit seldom performed presently^{57,64}. Given the use of advanced imaging in the diagnostics of both endocrine and neurological disease, a better understanding of the range of abnormalities encountered in the pituitary gland is desirable. At present, there is limited data in the veterinary literature on acquired pituitary abnormalities,

in addition the data that is available is complicated by some lack of consensus over terminology and classification.

Pituitary lesions can be subclinical or cause endocrine dysfunction (hypofunction or hyperfunction) or neurological signs due to local effects of an expanding mass ^{60,74}. In people, approximately 15% of pituitaries examined at autopsy contain small pituitary adenomas, mainly prolactin producing, with the vast majority being clinically silent ³⁷. Other pathologic entities such as pituitary cysts and metastatic tumours are thought to have a similar prevalence in people ^{43,44}.

In dogs, the incidence of Cushing's disease (pituitary dependent hyperadrenocorticism) is estimated at 1-2 cases per 1000 dogs per year ⁵⁷, and pituitary adenomas account for 25% of all intracranial neoplasia in middle-age or geriatric dogs ^{34,68}. In cats, pituitary tumours are considered uncommon, accounting for 9.3 per cent of all intracranial tumours ⁷¹. No data currently exists estimating the presence of occult pituitary lesions (so-called incidentalomas) in dogs or cats to contrast with that in humans. This is extremely important given the increasing use of advanced neuroimaging in clinical practice.

1.2.3 Proliferative lesions within the pituitary gland

There are a wide range of pituitary neoplasms, some classifications and definitions still need to be clarified and agreed upon in veterinary medicine. Pituitary adenomas, may be functionally active ("functional adenoma") if there is excessive hormonal secretion, either continuously or episodically, possibly resulting in clinical syndromes of hormone excess ⁶⁰. According to the World Health Organization (WHO) classification of tumours of the endocrine system in humans ⁹ and animals ³¹, *pituitary adenomas* are classified using immunohistochemistry for pituitary peptide expression: adrenocorticotrophic hormone (ACTH), melanin-stimulating hormone (MSH), growth hormone (GH), prolactin (PRL), thyroid-stimulating hormone (TSH), follicle stimulating hormone and luteinizing hormone (FSH/LH)^{9,31}. Immunolabelling of a hormone does not necessarily signify that it is secreted in a physiologically active form ¹³. Pituitary tumours that have no hormone immunoreactivity are designated as *null-cell adenomas*, and often reach considerable size (up to 4 cm) before showing neurological clinical signs due to the mass effect on brain or optic nerves ^{9,28,31}. So far,

only an alpaca with neurological signs and null-cell adenoma has been described in the veterinary literature²².

In people, pituitary adenomas are subdivided in macroadenomas (>1cm) or microadenomas depending on the size ^{13,37}. This terminology has been adopted by some veterinary pathology books ³¹ and several articles ^{43,45} and in contrast to others stating the cut-off being 0.5 cm ⁴². The *WHO classification of Tumours of the endocrine system of Domestic Animals* also uses both definitions on different pages, adding to the current confusion when describing or classifying pituitary lesions ³¹.

Corticotroph adenomas derived from the *pars distalis* or *pars intermedia* are reported to be common in dogs and infrequent in cats ⁵⁹. These neoplasms are composed of polyhedral cells with ACTH, POMC and MSH positive immunolabelling, which vary in intensity depending on the secretory functionality ⁶⁰, and are the most common cause of Cushing's syndrome in dogs ⁷⁰. *MSH-secreting adenomas* are rare, share similar microscopic characteristics with ACTH-secreting adenomas and present strong immunoreactivity for α -MSH, its precursor pro-opiomelanocortin (POMC), and a weaker ACTH-positive signal ^{23,39}. Human literature reports that corticotroph adenomas are periodic-acid Schiff (PAS) positive, attributed to glycolipids, glycoproteins or mucoproteins in the secretory granules or in their membranes ⁵². FSH, LH and TSH are glycoproteins thus their secretory granules also would stain with PAS, however, *thyrotroph* and *gonadotrophic adenomas* have not been reported in dogs and cats yet.

Somatotroph adenomas are acidophilic adenomas composed of PAS-negative cells that stain positive against GH ^{17,51}. Insulin resistant diabetes, acromegaly and hyperadrenocorticism might be caused by a functional adenoma in cats ^{12,24,49,66}, but this neoplasm is rarely reported in dogs ^{17,29,59}, where acromegaly is often related to GH hypersecretion of mammary origin ¹⁸. There is a recent description of a *lactotroph adenoma* in a male dog, with mammary gland swelling and galactorrhea and serum prolactin concentration markedly elevated, which histologically consisted of a monomorphic population of cells with small amount of pale cytoplasm, with some rosettes and presence of pre-existing acidophils ⁷.

Pituitary carcinomas: In people, the diagnosis of pituitary carcinoma requires the presence of distal or intracranial discontinuous metastases, or bone invasion ^{13,37,56}. This definition is also adopted in veterinary medicine by some main references

and articles ^{31,42,53,59}. The term “invasive or atypical adenoma” is used to refer to pituitary tumours that have a suprasellar extension or bone erosion without metastasising is broadly used ^{31,53}. However, a reference book and several articles consider pituitary carcinoma as the correct diagnosis for any aggressive pituitary invasive growth into the CNS or adjacent structures ^{30,34,55,60}, therefore this definition still needs further clarification in human and veterinary medicine. The prevalence of pituitary carcinomas by either definition is low in dogs ^{53,59}, with only few cases showing metastases ^{21,53,63}. One single case of pituitary carcinoma was reported in a cat with marked bone invasiveness but no metastases ³⁰. In people, pituitary carcinomas are normally non-functional despite the expression of pituitary peptides (ACTH or PRL) ¹³, and in dogs both silent and ACTH-secreting carcinomas have been observed ^{21,53}.

Craniopharyngioma is a benign multilobular tumour which occurs very rarely in young dogs and cats which derive from epithelial remnants of the oropharyngeal ectoderm and is positive in immunohistochemistry against cytokeratin ^{11,31,59}. In 2 middle-aged cats, the malignant counterpart of craniopharyngioma has been described, with presence of marked anaplastic features and infiltration into the surrounding bone ⁴⁷.

Pituitary hyperplasia is a controlled cell proliferation that is induced by a stimulus like lactation or hypothyroidism, which resolves once the stimulus is removed, and may be clinically indistinguishable from adenoma, therefore it falls to the pathologist to make the diagnosis: the reticulin stain can be used to differentiate it from adenoma: pituitary adenomas reveal total breakdown of normal acinar architecture on Gordon-Sweet reticulin stain, which distinguishes neoplasia from hyperplasia that retains an acinar reticulin pattern ^{31,41}.

The sellar region can also be affected by the presence of *metastatic tumours* from distant sites, such as malignant lymphoma, melanoma, transmissible venereal tumour and adenocarcinoma, or compressed by regional bone neoplasm ⁶⁰.

1.2.4 Pituitary cysts

A survey detected Rathke’s cleft cysts in 53% of dogs from several breeds, especially in brachycephalic breeds ^{59,65}, which occasionally become large enough to exert pressure on adjacent structures, or even disrupt pituitary function ⁵⁹. Cysts and cyst-like structures often

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contain PAS-positive, pale mucinous fluid and can be classified based on the lining epithelium and location following a previously proposed system ⁶⁵ : cysts with epithelial lining; cystic areas without an epithelial lining; and enlarged colloid follicles of the pars intermedia. The epithelial lining was a cuboidal, ciliated, single-cell layer epithelium in 3 dogs, and a non-ciliated, flat to cuboidal single cell-layer epithelium in the remaining cysts.

In young German Shepherds, Saarloos and Czechoslovakian wolfdogs, persistent cysts of the Rathke's pouch link to a mutation in LHX3, resulting in combined pituitary hormone deficiency and congenital dwarfism ⁷².

1.2.5 Hypophysitis

Primary inflammatory conditions of the pituitary gland are rare, often associated with autoimmune origin, and reported to occur much less commonly than primary tumours in human beings; also hypophysitis secondary to a systemic disorder may happen ^{5,36}.

1.3 Material and methods

1.3.1 Cases and histopathology

A total of 201 pituitary glands were sampled from routine necropsies from 136 dogs and 65 cats at the Veterinary Pathology Service at the Veterinary School (SVMS) of the University of Nottingham between 2015 and 2016. This was a convenience sampling study that resulted in a population of dogs with a median age of 6 y (2 - 8.5 y), with a range of 4 days to 14 y. The age distribution was as follows: 51 young (<4 y), 43 middle-aged (4-<8 y) and 42 old-aged ($\geq$ 8 y) dogs. Cats showed a median age of 9y (4 - 14 y), with a range of 1 month to 20 y. The age distribution was as follows: 14 young (<4 y); 12 middle-aged (4-<8 y) and 39 old-aged ($\geq$ 8 y). The proportion of males was 57% of the dogs and 55% of the cats. The neuter status for both genders was 42% and 72% of the dogs and cats respectively. The most common canine breeds submitted were Greyhounds (10%), Labrador retriever (10%) and Staffordshire terrier (7%), and 69% of the cats were domestic short/long hair. Cases were submitted as part of routine diagnostic investigations or were donated for teaching purposes. Individual age, gender, breed, clinical history and final diagnosis were recorded from each animal (Supplemental data 1.7).

Pituitary glands were immediately fixed in 10% neutrally-buffered formalin before performing a sagittal trimming of each gland into 2 halves for embedding both in paraffin wax. Tissue sections (5 μ m) were stained with haematoxylin and eosin (HE) for routine microscopic analysis. Measurement of the size of the lesions was performed on the section with the widest lesion-diameter after sectioning the paraffin block of the affected gland for all stains (3-9 sections per lesion).

All major endocrine glands were examined in all post mortem cases. These were only sampled for histopathological evaluation if macroscopic changes were identified or suspected.

1.3.2 Special stains and immunohistochemistry (IHC)

Tissue sections of thirteen (12 dogs and 1 cat) pituitary glands presenting proliferative changes, and one normal control pituitary were stained with periodic acid Schiff (PAS) and

Gordon and Sweet's reticulin stain using appropriate control tissues for validation of the stains.

These same samples were stained against adrenocorticotrophic hormone (ACTH). In three canine samples with neoplastic changes which were entire or partially non-immunoreactive for ACTH, further IHC against growth hormone (GH), alpha melanocyte stimulating hormone (α -MSH) and prolactin was also done. Immunohistochemistry was performed using primary antibodies (ACTH, 1:100 monoclonal antibody against ACTH, clone 2F6, Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, the Netherlands; α -MSH, 1:400 polyclonal rabbit antibodies to synthetic α -MSH, MZ111; Biomol International, Exeter, UK; GH, 1:5,000 rabbit anti-human antibody STH, N1561, Dako, Glostrup, Denmark; and prolactin, using a non-commercial polyclonal antibody against prolactin raised in rabbits; courtesy of Dr B. P. Meij, Utrecht, the Netherlands) in an indirect immunoperoxidase staining procedure, using the avidin-biotin-based technique (Vectastain ABC kit, Vector Laboratories, Burlingame, CA, USA) on 5 μ m pituitary sections (5 μ m) following previously published protocols^{7,58}: Slides were labelled by immunohistochemistry using a polymer-based detection system (EnVision + System Labelled Polymer-HRP Anti-mouse, Dako, EnVision + System Labelled Polymer-HRP Anti-rabbit, Dako). Tissue samples were dewaxed and rehydrated, and endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 30 minutes. The nonspecific links were blocked for 20 minutes with 1/20 goat serum diluted in TBS-BSA (TBS: 0.5M Tris Base, 9% NaCl, and pH 7.4, TBS-BSA 0.9% bovine serum albumin). Slides were incubated overnight with the primary antibody and subsequently, the sections were incubated for 30 minutes using the EnVision+ system and developed with a solution of 3-3'-Diaminobenzidine (DAB) (Vector Lab, Burlingame, California, USA). The slides were stained with haematoxylin for 12 seconds and mounted. 5-minute washes with TBS were performed twice between each step.

Three cases with hypophysitis were additionally stained with Giemsa and Gram to detect potential infectious agents (such as protozoa and bacteria). In one of these dogs, IHC was conducted in a commercial lab using antibodies against canine CD3 and CD20 to characterize the lymphocytic population.

1.4 Results

Pituitary lesions were identified in 36 out of 136 (26.4%) dogs and 10 out of 65 (15.3%) cats. Eighteen (18/136, 13.2 %) dogs and 8 (8/65, 12.3%) cats had pituitary cystic changes; 12 (12/136, 8.8%) dogs and 1 (1/65, 1.5%) cat showed primary pituitary neoplasms, 2 (2/136, 1.4%) dogs had metastases of an extrasellar carcinoma (salivary adenocarcinoma and nasal adenocarcinoma), and 4 (4/136, 2.9%) dogs and 1 (1.5%) cat presented inflammatory lesions (Table 1). The remaining pituitary samples from 100 dogs and 55 cats were macroscopically and histopathologically unremarkable. The distribution of pituitary lesions in dogs based on the age is shown in Figure 3. All the cats presenting pituitary cysts were older than 8 y.

Table 1 Summary of pathological finding in the pituitary glands

Pathological findings	Dogs		Cats	
	Number	%	Number	%
Pituitary cysts	18	13.2	8	12.3
Pituitary neoplasia/hyperplasia	12	8.8	1	1.5
Pituitary adenoma	11	8.0	0	0
Pituitary carcinoma	1	0.7	0	0
Pituitary hyperplasia	0	0	1	1.5
Inflammation	4	2.9	1	1.5
Metastases	2	1.47	0	0
No lesion	100	73.5	55	84.6

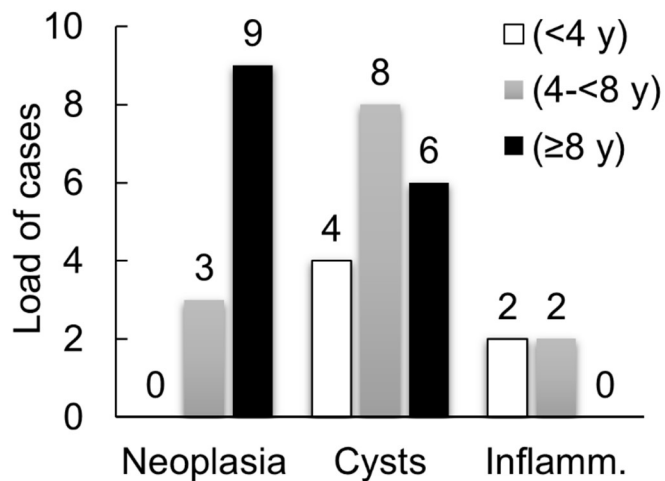


Figure 3 Distribution of pituitary lesions (pituitary neoplasia, cysts/cyst-like lesions and inflammation) in dogs by age ranges of <4 years (white column); 4-<8 y years (grey column); >8 years (black column). Only dogs with lesions are included in this plot.

1.4.1 Pituitary neoplasia and hyperplasia

Affected dogs were of various ages (range, 4 to 15 y; median 10 y), with a prevalence in middle aged and old dogs of 7% (3/43) and 21% (9/42) respectively; these were of both genders and various breeds (3 Greyhounds, 1 whippet, 1 Lhasa Apso, 1 poodle, 1 Cocker spaniel, 1 German Shepherd, 1 Retriever Labrador, 1 Bull Mastiff crossbreed, 1 Dachshund crossbreed, and 1 Leonberger). Only a 23-year-old domestic short hair cat showed primary pituitary hyperplasia.

Pituitary adenoma. Only one dog of the 11 that displayed a pituitary adenoma, a Dachshund crossbreed, had been diagnosed with hyperadrenocorticism ante mortem, and showed typical associated skin and hepatic lesions. Adrenal gland tissue for histopathological examination was available from this dog and three others of the 11 pituitary adenoma dogs, and all presented moderate bilateral adrenocortical hyperplasia. In a further 33 dogs with no pituitary proliferative change, 10 (30%) dogs also showed bilateral adrenal cortical hyperplasia. No other systemic lesions compatible with hyperadrenocorticism were observed in the remaining 7 (63.6%) dogs.



Figure 4 Pituitary adenoma, dog.

Most adenomas were over 2 mm in size (7/11, 63%), with a mean size of 3.2 mm, but only 2 (18%) pituitary adenomas were detected grossly as bulging, soft, non-encapsulated, pale, nodules of 7.0 mm and 5.4 mm in maximum diameter, which caused mild pituitary enlargement and loss of symmetry of the dorsal aspect of the gland with no invasion of the adjacent structures (Fig. 4). The adenoma in the dog in which hyperadrenocorticism was established ante mortem was 1.8 mm in maximum diameter. Microscopically, all adenomas were located in the pars distalis and shared a similar appearance, consisting of variably demarcated, densely cellular nodules of neoplastic cells (Fig. 5 a), arranged in sheets (diffuse pattern) with fine supportive stroma, and loss of the normal stromal network pattern shown in the reticulin stain (Fig. 5 b). Neoplastic cells showed little pleomorphism with small to medium-sized nuclei, coarse chromatin pattern, and very characteristic abundant, light eosinophilic in HE (Fig. 5 a), uniform cytoplasm that contained PAS positive granules (Fig. 5 c), which consistently corresponded to an ACTH immunoreactive signal when IHC was performed (Fig. 5 d). No mitotic figures or areas of necrosis were observed in any case. In addition to the described neoplastic cells, in the two largest corticotroph adenomas, there were cells that did not contain PAS positive cytoplasmic granules and that were not immunoreactive to ACTH. These cells had a lower amount of cytoplasm devoid in PAS positive or ACTH immunoreactive secretion granules, and a round to oval nuclei of coarse chromatin, supported by fine fibrovascular stroma. These cells were also non-immunoreactive to MSH, GH or PRL immunohistochemistry.

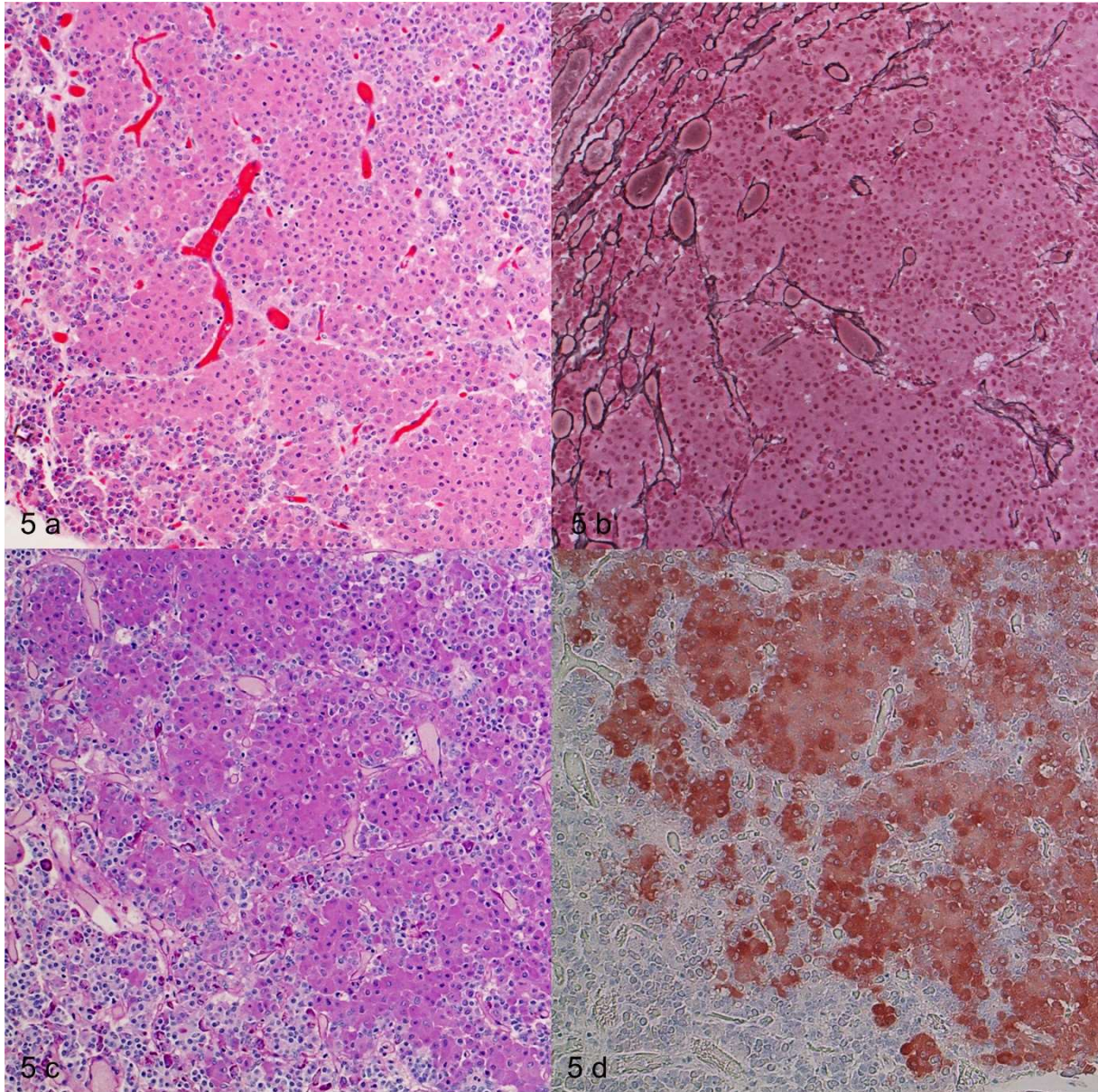


Figure 5 Pituitary adenoma, dog, with loss of normal reticulin stromal pattern, HE x20 (a), reticulin stain x20 (b), abundant, violet, uniform, PAS positive cytoplasm, PAS x20 (c) and strong ACTH immunoreactivity, ACTH-IHC x20 (d).

Within the pars distalis and adjacent to 4 of the pituitary corticotroph adenomas, there were small numbers of secretory cells that show margination of intracytoplasmic PAS-positive and ACTH immunoreactive granules (Fig. 6a), leaving a clear paranuclear halo. These cytoskeletal changes showed immunolabelling with pan-cytokeratin IHC (Fig. 6b).

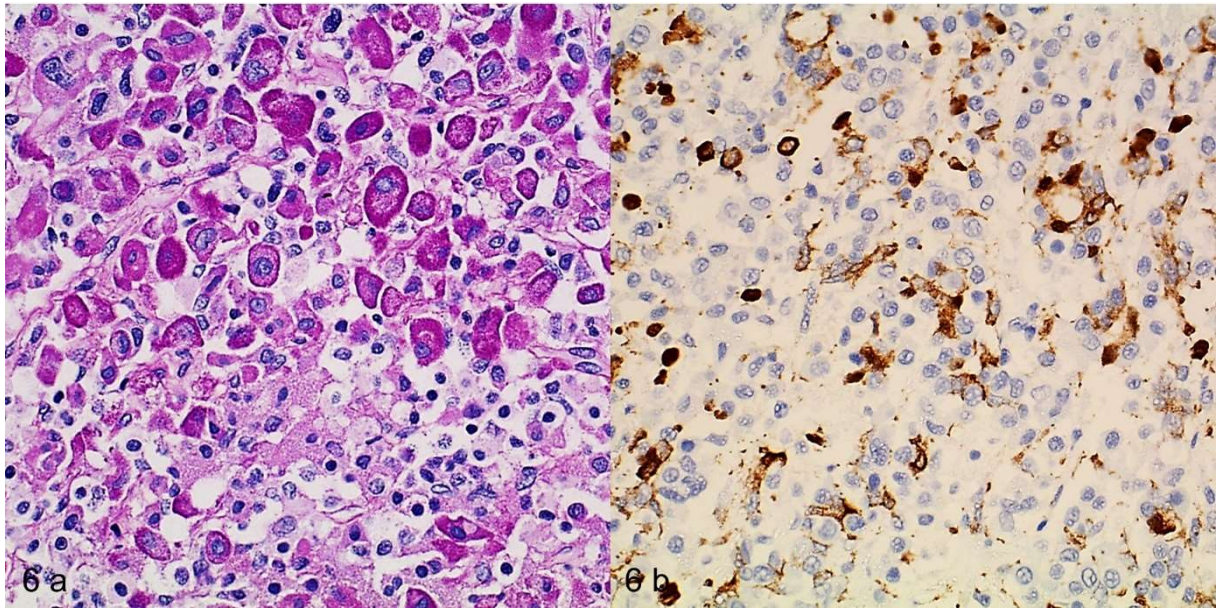


Figure 6 Pituitary adenoma, dog, clusters of non-neoplastic corticotrophs with intense PAS positive granules in the periphery with clear paranuclear stain, PAS x40 (a), and some cells show perinuclear pancytokeratin immunoreactivity, PanCK, IHC x 40 (b).

Pituitary carcinoma. A 7-year-old, male greyhound had a pituitary carcinoma manifesting neurological signs, neck pain and low head carriage without signs associated with an endocrinopathy. On post-mortem examination, a 2 x 3 x 2 cm, non-encapsulated mass arose from the pars distalis infiltrating and markedly invading dorsally and discontinuously into the hypothalamus and thalamic region. Histologically, polygonal cells with distinct cell borders and round nuclei were arranged in tightly-packed cords, islets or nests, separated by moderate amounts of fibrous stroma (Fig. 7a and 7b), and infiltrating the neurohypophysis and diencephalon that also showed moderate gliosis (Fig. 8). Cellular pleomorphism was moderate and there were 4-5 mitotic figures per 400x field with multiple necrotic foci which often had a central core of dystrophic calcification. PAS stain (Fig. 7 c) and ACTH, MSH, GH, and PRL-IHC were negative (Fig. 7 d).

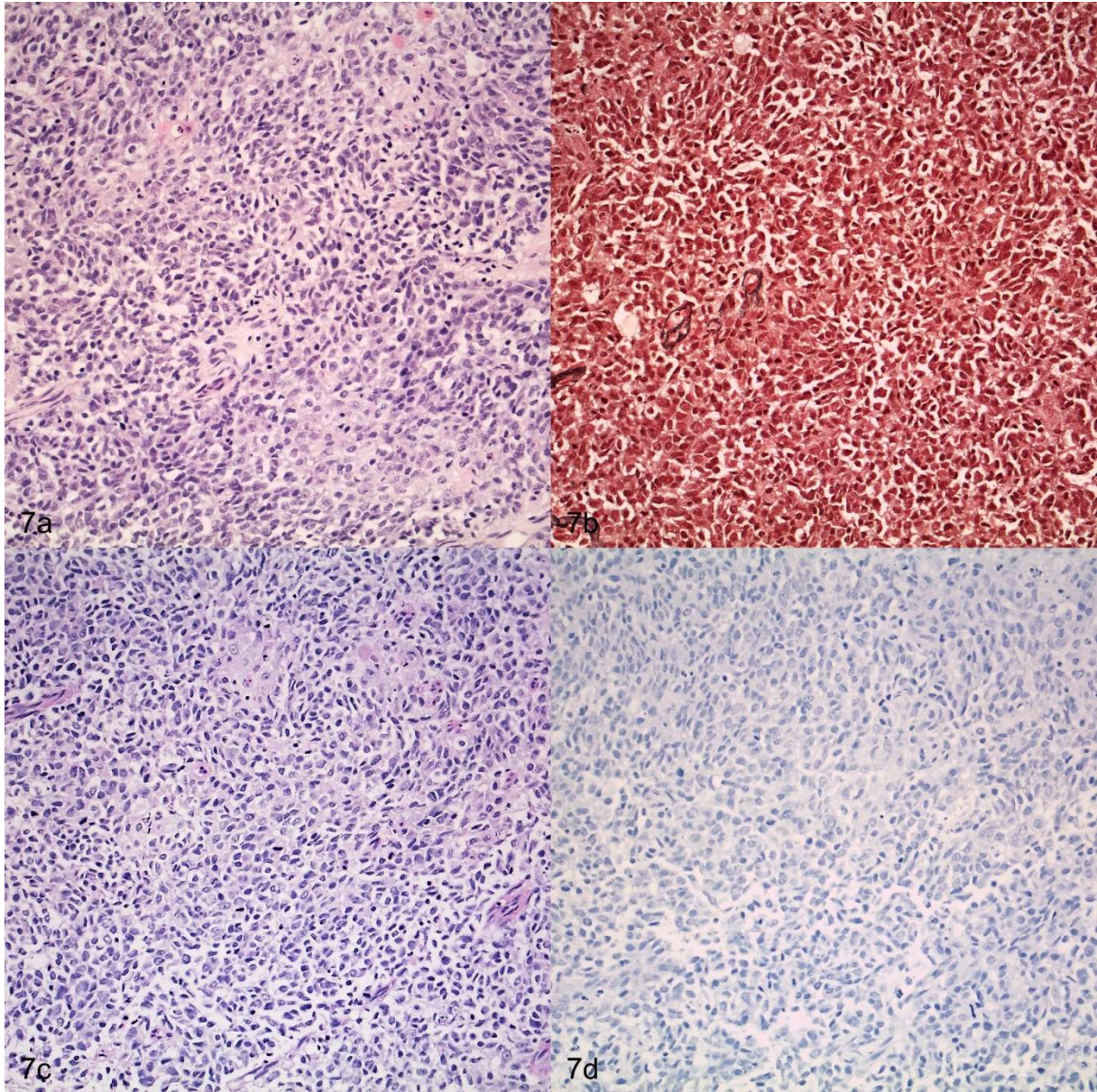


Figure 7 Pituitary carcinoma, dog, HE x20 (a), reticulin stain x 20 (b), PAS negative stain x20 (c), ACTH non-immunoreactivity x20 (d).

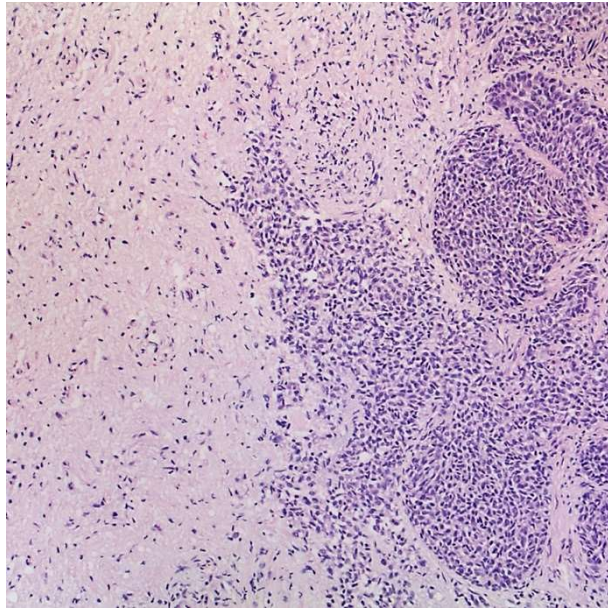


Figure 8 Pituitary carcinoma, dog, infiltrating dorsally the thalamus, HE x10.

Pituitary hyperplasia. One cat presented focal nodular pituitary hyperplasia within the pars distalis, without any previous manifestation of clinical signs or presence of systemic lesions suggestive of hyperadrenocorticism (or hyperthyroidism). Histologically, there was a well-circumscribed, 1.4 mm in diameter, focal, non-encapsulated, nodular proliferation of secretory cells which caused enlargement of the acini (Fig. 9a), mildly compressing the adjacent pars distalis, with minimal to no visible changes in the normal pituitary stromal network in the reticulin stain (Fig. 9b). Hyperplastic cells revealed a chromophobic cytoplasm, often vacuolated and poorly stained with PAS, with a negative or very weak ACTH-immunolabelling.

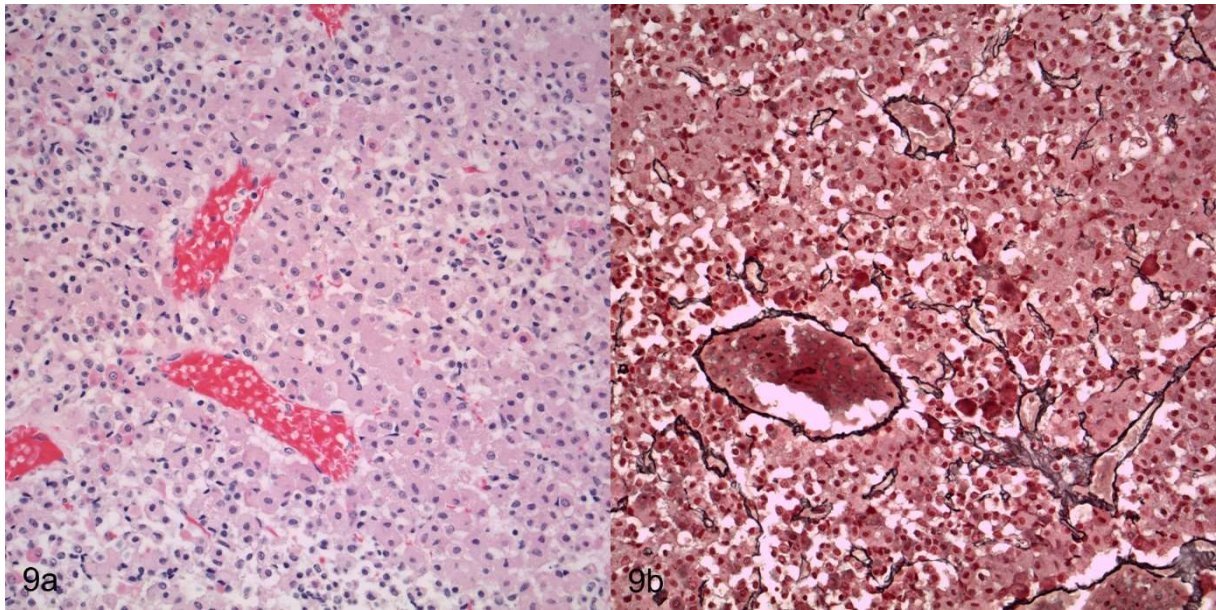


Figure 9 Pituitary hyperplasia, cat, HE x20 (a), with minimal to no visible changes in the normal pituitary stromal network in the reticulin stain x20 (b).

Secondary metastases. Two (1.5%, 2/136) unrelated Border collie dogs of 10 and 9 y had intracranial metastases from a nasal adenocarcinoma and salivary adenocarcinoma respectively, which also affected the pituitary gland, the former destroying the gland entirely and the latter infiltrating and replacing approximately 50% of the *pars distalis*. Clinically, both these dogs showed prominent neurological signs with no signs of an associated endocrinopathy.

1.4.2 Pituitary cysts

Eighteen (13.2%) dogs from various ages and breeds and 8 (12.3%) old cats (mostly domestic short hair), with a median age of 7 y (5-9 y) and 11 y (8.5-14 y) respectively, showed pituitary cystic lesions without any related clinical signs reported in the clinical history (endocrine investigations had not been performed in these cases). Eight dogs out of the 18 (44%) were brachycephalic breeds.

Cysts were variable in size up to 2.7 mm, with a mean diameter of 1.6 mm in dogs, and up to 0.8 mm in cats, with a mean diameter of 0.4 mm. Cysts and cyst-like structures contained PAS-positive, pale mucinous fluid and were morphologically classified based on the lining epithelium and location following a previously proposed system⁶⁵ (Fig. 10): cysts with

epithelial lining detected in 10 dogs and 4 cats; cystic areas without an epithelial lining seen in 7 dogs and no cat; and enlarged colloid follicles of the pars intermedia observed in 1 dog and 4 cats. The epithelial lining was a cuboidal, ciliated, single-cell layer epithelium in 3 dogs, and a non-ciliated, flat to cuboidal single cell-layer epithelium in the remaining cysts.

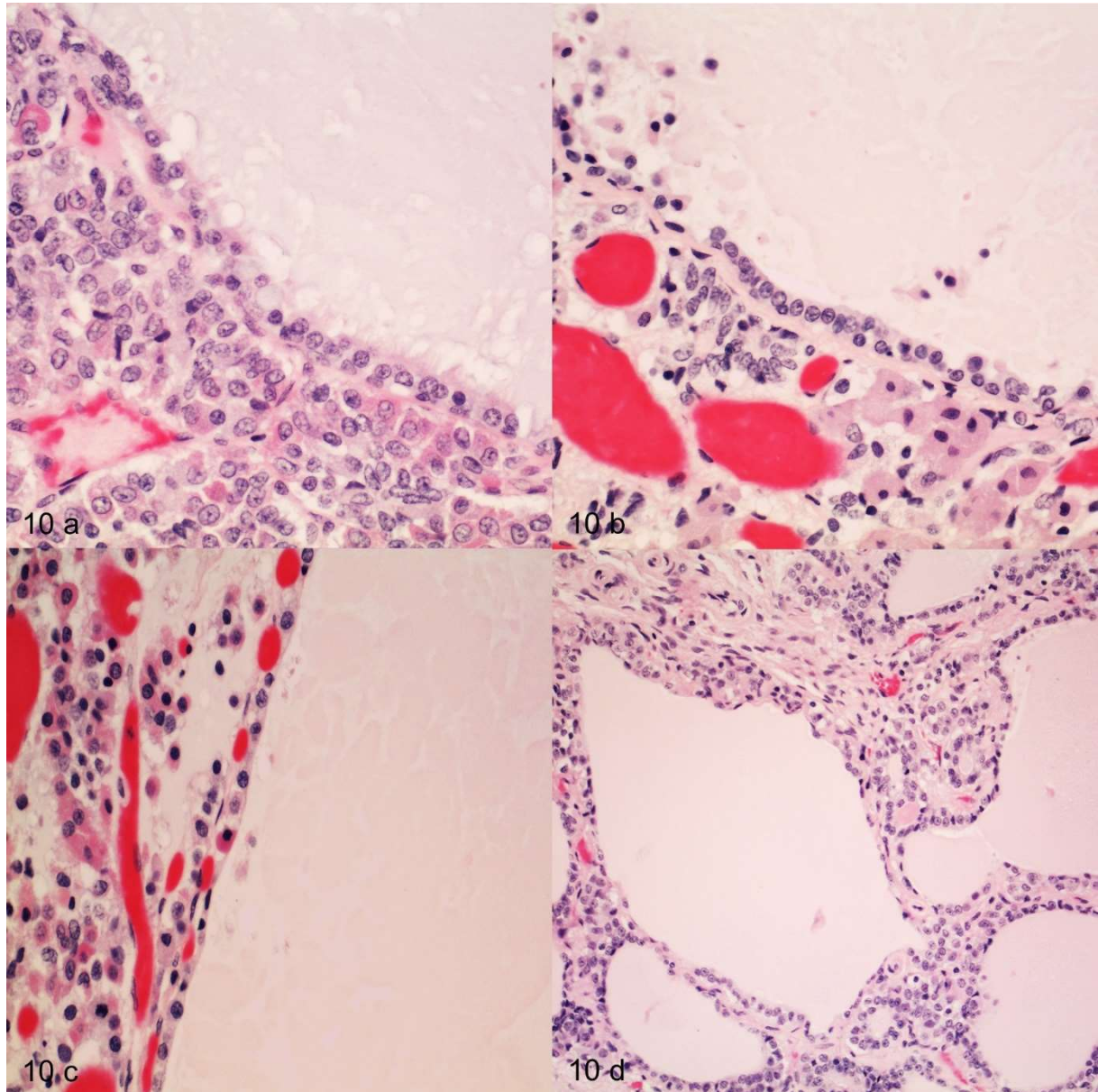


Figure 10 Pituitary cyst, dog, containing, pale mucinous fluid lined by ciliated epithelium, HE x40 (a). Pituitary cyst, dog, containing, pale mucinous fluid lined by cuboidal, non-ciliated epithelium, HE x40 (b). Pituitary cystic area, pars distalis, dog, without an epithelial lining, HE x40 (c). Enlarged colloid follicles, cat, pars intermedia, HE x40 (d).

1.4.3 Inflammatory lesions

Two dogs, a male 6-year-old Scottish terrier (further information in 1.8) and a male 6-year old Lurcher, had hypophysitis accompanied by moderate bilateral cortical atrophy of the adrenal glands and clinically presenting with Addison's syndrome. Both cases showed a moderate to severe, lymphoplasmacytic inflammatory infiltration with loss of pituitary cells. The observed lymphocytic infiltrate was predominantly T-cell rich with low numbers of B cells in the dog where IHC was performed. No evidence of microorganisms or foci of inflammation were detected in the brain or other tissues.

Secondary hypophysitis was observed in two other dogs, one presenting non-suppurative encephalitis caused by canine distemper virus (extending into the neurohypophysis) and the other as part of an ante mortem confirmed systemic immune-mediated disease comprising immune-mediated polyarthrits, vasculitis and anaemia. One cat presenting with feline infectious peritonitis (FIP) showed perivascular neutrophilic and lymphoplasmacytic infiltrate within the neurohypophysis.

1.4.4 Other findings

One (0.7%) 1-year-old Vizsla dog and 8 (15.4%) cats (range 2 to 20 y, median age 10.5 y) showed multifocal areas of mineralization in otherwise unremarkable pituitary glands. The dog presented with chronic renal failure with metastatic calcification affecting various organs, including multifocal, poorly-defined mineralization affecting the neurohypophysis. The cats did not have a clinical history or lesion compatible with hypercalcemia and revealed very few (1 to 3), scattered, up to 200 µm in diameter, round, mineral concretions with onion- skin like shape within the pituitary capsule, in the neurohypophysis or within acini in the adenohypophysis.

Pituitary glands from all 23 puppies and young dogs (up to 9-month-old) and a lactating bitch, showed a uniform appearance of the adenohypophysis, revealing a more basophilic cell appearance with cytoplasm devoid of granules, and large, vesicular nuclei compared to our adult or elderly population of dogs (Fig. 11). This difference was not

observed in 5 kitten/young cats (less than 9 months).

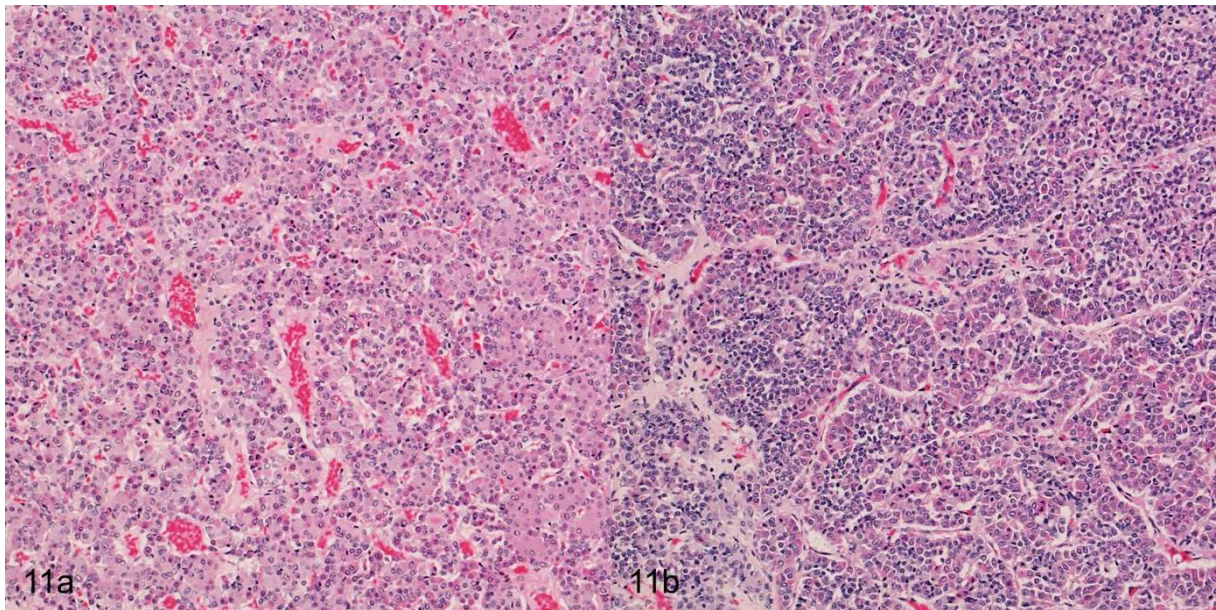


Figure 11 Normal pituitary gland, pars distalis, adult dog, HE x10 (a). Normal, pituitary gland, 6 weeks puppy, HE x 10 (b). Note the diffuse more basophilic appearance of the pars distalis, with less pituitary cells containing eosinophilic granules, and larger proportion of chromophobes or pituitary cells with cytoplasm devoid in granules.

1.5 Discussion

Within our study, pituitary abnormalities were frequent in both dogs (26.4%) and cats (15.3%); but only 4 dogs (4/136, 2.9%) were diagnosed with a clinical manifestation of pituitary disease, therefore the presence of a pituitary lesion in most of our cases is unexpected; very similar to the reports in people with incidentally observed pituitary lesions.

1.5.1 Neoplastic and hyperplastic lesions.

Pituitary neoplasms were more common in dogs than in cats in the studied population, particularly dogs older than 6 years, agreeing with the literature ⁵⁹. In our study, a total of 4 greyhounds and greyhound-cross breeds presented pituitary neoplasia that might suggest a higher susceptibility of this breed to this type of neoplasia, but a further study with a larger dog population is needed to confirm this.

The most frequently found neoplasm in our canine population was the ACTH-immunoreactive pituitary corticotroph adenoma, with a prevalence of 7% in middle aged and 21% in old dogs. Clinical signs consistent with pituitary disease were not reported ante mortem in ten (10/11, 90.9%) of the dogs presenting with pituitary adenoma which may be explained by a lack of hormonally active ACTH secretion in otherwise immunohistochemically immunoreactive corticotroph adenomas. In people, they are thought to arise from corticotrophs that fail to process the ACTH precursor, pro-opiomelanocortin, into the biologically active 1–39 ACTH ³.

The presence of diffuse, moderate adrenocortical hyperplasia that might suggest an increased production of ACTH was seen in all dogs with pituitary adenomas where adrenal gland tissue was available; however, adrenal cortical hyperplasia was also observed in 30% of dogs without pituitary adenomas or hyperplasia. Thus, without measuring serum cortisol or ACTH and performing relevant dynamic functional tests, an association between these lesions must be considered with caution in dogs. Nodular adrenocortical hyperplasia is relatively common in older dogs without a detectable pituitary lesion and often attributed to an increased hypothalamic catabolism of dopamine that disrupts negative feedback control in the hypothalamic-pituitary-adrenal axis ^{42,46}.

Only 1 dog had been diagnosed ante mortem with hyperadrenocorticism and showed typical lesions of pituitary dependent hyperadrenocorticism; with a very small (1.8 mm in diameter) ACTH-immunoreactive pituitary adenoma, which did not cause any grossly observable enlargement of the pituitary gland. It has been reported that pituitary adenomas less than 1 cm are more likely to be functional than larger ones ³¹.

In our opinion, the significance of the rather arbitrary term micro/macroadenoma based on a cut-off limit of 0.5 or 1cm to indicate a possible mass effect, used in human medicine ^{13,37} and adopted in veterinary pathology books ³¹ and some articles ^{43,45}, is questionable since it does not take into account the variation in size of the pituitary glands in different species and differently-sized animal breeds. Radiologists expect to see lesions from 2-3 mm in diameter on upwards on MRI, depending on the resolution of the device used, and improved technical equipment and increased usage of medical imaging will increase numbers of lesions identified in pituitary glands of small animals. It might prove to be more useful therefore for the communication between pathologists and clinicians to use the term *macroadenoma* to indicate a visible enlargement of the gland on diagnostic imaging or on post-mortem examination, and to include the size of the neoplasm when possible.

Histologically, every pituitary adenoma gave a well-defined, strong, uniform, intracytoplasmic ACTH immunereaction, and presented an almost identical histological morphology: abundant, granular, eosinophilic, cytoplasm which stained uniformly violet with PAS which corresponded consistently to ACTH immunolabelling. Human literature reports that corticotroph adenomas show PAS positive intracytoplasmic granular staining, attributed to glycolipids, glycoproteins or mucoproteins in the secretory granules or in their membranes ⁵². In pathological diagnostic settings in which IHC is not available, a strong cytoplasmic granular PAS positivity in a proliferative area with a loss of reticulin fibres can support a diagnosis of ACTH-corticotroph adenoma, bearing in mind that cells producing TSH and FSH/LH also contain PAS-positive granules (but so far these latter neoplasms have not been reported in dogs) ³¹. MSH-secreting adenomas share similar microscopic characteristics with ACTH-secreting adenomas but present strong immunoreactivity for α -MSH, its precursor pro-opiomelanocortin (POMC), and are weakly ACTH-immunoreactive ^{23,39}.

The ACTH non-immunoreactive neoplastic population with scant cytoplasm observed within 2 corticotroph adenomas might have been corticotroph cells that have already released their secretory granules. However, despite seeming less likely, a pre-existing, non-neoplastic cell population (suggesting infiltrative growth) or a second neoplastic population of FSH/LH or

TSH- secreting cells cannot be ruled out ⁷⁵. Unfortunately, we were unable to identify a source of available antibodies for these additional hormones in dogs and cats.

In people with functional ACTH-secreting pituitary proliferations, non-neoplastic corticotrophs can be encountered that show ACTH immunoreactive secretory granules pushed to the periphery of the cytoplasm by homogeneous, hyaline material consisting of cytokeratin microfilaments arranged concentrically around the nucleus. This morphological pattern is known as Crooke's hyaline degeneration and these cells are called *Crooke's cells*. It is a characteristic morphological finding in the functional version of ACTH immunoreactive neoplasms in humans ^{9,41,50}, which to our knowledge has not been described in small animals yet. The few nonneoplastic corticotroph cells with strong, PAS-positive, peripheral, intracytoplasmic staining seen adjacent to 4 canine corticotroph adenomas in our study population, morphologically resemble these so called *Crooke's cells* with peripheral dispersion of PAS positive granules in the cytoplasm and a strong cytokeratin immunolabelling. However, the peripheral hyaline bands appeared to not be easily identifiable on HE but cytoplasmic accumulation of intermediate filaments could be demonstrated on cytokeratin IHC (Fig. 4b).

The reticulin stain turned out to be very useful to differentiate adenomas from pituitary hyperplasia when diagnosis was doubtful by highlighting the pre-existing reticulin network: pituitary adenomas reveal absence of normal acinar architecture in a Gordon-Sweet reticulin stain, which distinguishes neoplasia from hyperplasia which retains an acinar reticulin pattern ^{31,41}. However, pituitary adenomas and hyperplasia appear to be a continuous spectrum of proliferative lesions between focal (nodular) hyperplasia and adenomas, with a similar prognosis and treatment ⁵⁹, so distinction between both entities seems merely academic. This stain also helped to avoid over interpretation of proliferative lesions, especially in cats as their normal pituitary appearance is less homogeneous than in dogs, and exhibits great variation in cell populations, which apparently correlates with certain phases of the reproductive cycle ⁸.

We considered the infiltrative pituitary mass presented in this study a pituitary carcinoma because of the observed necrosis, presence of mitoses, invasion and widespread, destructive, discontinuous growth into the brain, following other authors guidance ⁶⁰. However, we are aware of the discrepancy with other references where metastatic behaviour of the tumour is needed to diagnose a pituitary carcinoma ^{31,42,53,59}, and the term "invasive or atypical adenoma" is used instead ^{31,53}. As a general criterion, destructive infiltration into a neighbouring organ is considered a reliable feature that differentiates cancers from benign

tumours ^{33,48}, thus we find the term “pituitary carcinoma” more appropriate for those cases where there is marked invasion and destruction of the brain. The prevalence of pituitary carcinomas by either definition is low in dogs ^{53,59}, with only few cases showing metastases ^{21,53,63}, and only one single case of pituitary carcinoma was reported in a cat with marked bone invasiveness but no metastases ³⁰.

In the 2 cases of pituitary metastases from a salivary adenocarcinoma and nasal adenocarcinoma of the current study, the observed neurological signs were mainly attributed to other co-existing intracranial metastases from the same primary neoplasm. Ante mortem clinical signs of pituitary dysfunction were not detected, similar to what is reported in people where only 7% of the metastases to the pituitary are symptomatic, being diabetes insipidus, visual field defects, headache/pain and ophthalmoplegia the most commonly reported symptoms ¹⁶. Distant metastases from extrasellar carcinomas to the pituitary gland such as lymphoma, melanoma, transmissible venereal tumour and adenocarcinomas have been previously reported ⁵⁹.

1.5.2 Pituitary cysts

Pituitary cysts or cyst-like lesions were the most common finding, affecting dogs and cats similarly (12-13%), and appear to be a more common finding in middle-age to old dogs and elderly cats. The cysts with epithelial lining, sometimes ciliated, are thought to be remnants of the distal craniopharyngeal duct inside Rathke’s pouch, while the cystic areas without epithelial lining appear to arise from degeneration of pituitary tissue or blockage of secretion ⁶⁵. Enlarged colloid containing follicles of the pars intermedia were found more often found in cats. All pituitary cysts found in this study were considered subclinical, but occasionally cysts may become large enough to exert pressure on adjacent structures, or even disrupt pituitary function ⁵⁹.

In the current study brachycephalic breeds appeared to have a higher prevalence of pituitary cysts than non-brachiocephalic breeds as previously identified; however, the prevalence we observed in dogs in general is markedly lower compared to a previous survey where cystic craniopharyngeal duct remnants were detected in approximately 50% of the dogs ^{59,65}. This previous survey limited the studied population to 6 breeds (Dachshunds, Terriers, Schnauzers, Boxers, French Bull dogs and German shepherd, no data are available about the proportions of each breed), whereas we included dogs from 58 breeds. If in the previous study

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the proportion of brachycephalic dogs was higher, this might explain the differences in prevalence.

1.5.3 Inflammatory lesions:

Two dogs and one cat presented hypophysitis secondary to systemic disease: canine distemper virus infection, clinically confirmed autoimmune disease with polyarthritis and vasculitis, and feline infectious peritonitis. The other two canine cases of hypophysitis appeared to be primary and we hypothesized immune-mediated in origin, because no other focus of inflammation or infectious agents were detected in these cases. Only a few cases of autoimmune lymphocytic adenohypophysitis have been reported in dogs so far ^{1,38,40,54,61,73}, usually related to panhypopituitarism and secondary hypoadrenocorticism with adrenocortical atrophy, similar to the cases identified here.

1.5.4 Other findings

Multifocal mineralization of the pituitary gland seems to be more common in cats (15.4%) than in dogs (0.7%), and in all 8 cats it was considered to be an incidental finding ¹³. In the affected dog, the mineralization was considered to represent metastatic calcification due to a calcium/phosphate imbalance related to chronic renal failure. To our knowledge, calcification in the pituitary gland has only been described previously together with neoplastic lesions or cysts ³², similar to the dystrophic calcification reported here in the necrotic areas of the pituitary carcinoma.

The uniform, more basophilic appearance of the pituitary gland in puppies, young dogs and a lactating bitch is interpreted as a diffuse, physiologic hyperplasia in highly active hormonal situations like growth or lactation. Additional immunohistochemical evaluation of the cell populations in the pituitary glands of these cases would be needed to substantiate this interpretation. In people, there is a progressive change in the appearance of the pituitary gland during the first years of life, according to the ongoing change in hormonal secretion ¹⁰. In humans, ageing changes such as fibrosis, deposition of amyloid, iron pigment and a decrease in acidophilic and chromophobic cells has been reported ^{27,62}, but none of these changes were consistently observed in geriatric dogs or cats we examined.

1.6 Conclusions

To summarize, given the presence of pathological changes in 26% of the dogs and 15% of the cats in our study, pituitary abnormalities should be considered common in both species. This is very much in keeping with the scenario described in humans. Most of these lesions were not identifiable macroscopically, so post-mortem sampling and histopathological examination of the pituitary gland is needed to avoid missing any pituitary abnormality. Within our population, we identified a prevalence of adenomatous pituitary lesions in middle aged to old dogs of 14% similar to the 10-15% reported in people ^{19,43}. The prevalence in cats appears to be much lower. Given that these lesions were incidentally observed in this cohort of dogs and cats, further investigations are needed to establish which are subclinical or which may have manifested clinically but were unnoticed on examination or anticipated during diagnostic investigations. A further prospective study incorporating the comprehensive clinical details of the dogs and cats submitted for necropsy would help determining their true clinical significance and clarify how incidental they really are.

1.7 Further work

After performing this work, several questions still come to our minds and also probably to the reader's. Some could not be solved because of the small population we worked with or the limited clinical ante mortem data we had. Also, funding for this project was restricted with only having a small budget of for histological processing, special stains and immunohistochemistry which limited the possibility of further tests.

Given this, there are several points that with more funding and with widened collaboration of clinicians and endocrinologists will be very interesting to address in the future:

- A larger standardised canine population will allow the use of statistics to estimate the real prevalence.
- A study comparing MRI imaging with histologically diagnosed adenomas could improve pre mortem diagnoses of pituitary adenomas/hyperplasia and therefore improve therapeutic interventions and life quality and prognosis of affected dogs and cats.
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- How functional or non-functional are most of this pituitary adenomas in middle/old aged dogs? A study of hormonal data from affected animals can give an answer to this question.
- If ACTH-immunoreactive adenomas are proven to be mostly non-functional, is there secretion of abnormal ACTH hormone that do not cause hormonal effect? Proteomic tissue and serum studies will be needed in this case.
- In cats, hyperthyroidism of unknown origin is relatively common, is it possible that the pituitary gland has a fundamental role? A comparison of TSH, T3, T4 levels, and a histopathological study of the thyroids and pituitary glands in normal cats and hyperthyroid cats could be performed in the future.

1.8 Supplemental Data

Table 2. Signalment of the cases.

ID	Species	Age	Breed	Gender	Diagnosis	Final diagnosis (Cause of death)
1338	D	6	AMERICAN BULLDOG	M	No lesion	Spinal cord herniation
1358	D	4	STAFFORDSHIRE BULL T.	F	No lesion	Euthanasia - Aggression
1378	D	1	LABRADOR RETRIEVER	M	No lesion	Bacterial pneumonia
1392	D	6	DOBERMAN	M	No lesion	Dilated cardiomyopathy
1395	D	12	COLLIE	M	Cysts	Intracranial meningioma
1397	D	7	COLLIE	F	Cysts	Malignant extramedullary plasmacytoma
1404	D	5	CHIHUAHUA	F	No lesion	Unknown
1405	D	16	STAFFORDSHIRE x	M	No lesion	Liver rupture-Hypovolemia
1408	D	7 M	BRITISH BULL DOG	F	No lesion	Unknown
1412	D	2 W	FRENCH BULLDOG	M	No lesion	Unknown
1413	D	4.5 M	IRISH WOLFHOUND	F	No lesion	Bacterial pneumonia - Sepsis
1414	D	7	AMERICAN BULLDOG	M	No lesion	Euthanasia - Aggression
1436	D	8 M	GERMAN SHEPHERD	F	Hypophysitis	Canine distemper
N15-055	D	10	LURCHER	F	No lesion	Bacterial pneumonia - Sepsis
N15-099	D	6 M	BASSET HOUND	F	No lesion	Pyothorax - Bacterial cellulitis
N15-100	D	10 M	LABRADOR RETRIEVER	M	No lesion	Bacterial pneumonia - sepsis
N15-102	D	4	GREYHOUND	M	No lesion	Possible cardiac failure

N15-133	D	6	AMERICAN BULLDOG	M	No lesion	Euthanasia - Aggression
N15-182	D	1	LABRADOR RETRIEVER	F	No lesion	Cardiac failure
N15-185	D	12	COLLIE	F	No lesion	Nasal adenocarcinoma
N15-188	D	9	LABRADOR RETRIEVER	M	No lesion	Dilated cardiomyopathy
N15-200	D	5	STAFFORDSHIRE BULL T.	F	Cysts	Euthanasia - Aggression
N15-213	D	2	SIBERIAN HUSKY	M	No lesion	Euthanasia - Aggression
N15-215	D	5	YORKSHIRE TERRIER	F	No lesion	Unknown
N15-236	D	3	GOLDEN RETRIEVER	M	No lesion	Unknown
N15-245	D	11	CROSSBREED	F	No lesion	Anaplastic mammary carcinoma
N15-251	D	7	POODLE	M	Cysts	Unknown
N15-255	D	11 M	LABRADOR RETRIEVER	M	No lesion	Haemorrhagic pneumonia
N15-256	D	15 M	BULLDOG	F	No lesion	Dilated cardiomyopathy
N15-259	D	11	WEST HIGHLAND WHITE T.	F	No lesion	Cardiac acute necrosis
N15-261	D	6 W	CROSSBREED	M	No lesion	Haemorrhagic enteritis
N15-268	D	3	BORDER COLLIE	F	No lesion	Septicaemia
N15-269	D	5	BRIARD	M	No lesion	Massive hepatic necrosis
N15-273	D	7	YORKSHIRE TERRIER	M	No lesion	Intestinal volvulus
N15-276	D	7	ENGLISH BULLDOG	F	Cysts	Brachiocephalic syndrome
N15-277	D	3 M	JACK RUSSELL	F	No lesion	Cerebellar atrophy

N15-280	D	8 W	FOXHOUND	M	No lesion	Myelinopathy
N15-283	D	9	STAFFORDSHIRE BULL T.	M	No lesion	Oligodendroglioma
N15-289	D	12	LHASA APSO	M	Adenoma	Bacterial haemorrhagic enteritis
N15-291	D	8	LABRADOR RETRIEVER	M	No lesion	Neospora cerebellitis
N15-298	D	2	CROSSBREED	M	No lesion	Pyothorax - Nocardiosis
N15-301	D	Adu lt	IRISH WOLFHOUND	F	No lesion	Cardiac failure - Severe endocardiosis
N15-303	D	2	GERMAN SHEPERD	F	No lesion	Haemoabdomen
N15-304	D	3	AMERICAN BULLDOG x	M	Cysts	Starvation
N15-311	D	9	CAVALIER KING CHARLES	M	No lesion	Cardiac failure- Endocardiosis
N15-320	D	6	POODLE	M	No lesion	Trauma neck
N15-322	D	10	WHIPPET	F	Adenoma	Multiple thrombosis
N15-327	D	3	GREYHOUND	M	No lesion	Asphyxia
N15-340	D	7	FLAT COATED LABRADOR	M	No lesion	Nervous histiocytic sarcoma
N15-342	D	6	SCOTISH TERRIER	M	Hypophysitis	Lymphocytic hypophysitis/hypothalamitis
N15-345	D	6	STAFFORDSHIRE BULL T.	F	No lesion	Acute pancreatic necrosis
N15-356	D	9	CROSSBREED	F	No lesion	Mammary carcinoma
N15-358	D	3	MALINOIS	M	No lesion	Euthanasia - Aggression
N15-366	D	3	GREYHOUND	M	No lesion	Gastric torsion
N15-369	D	4 M	VIZSLA	M	No lesion	Metastatic calcification
N15-373	D	8 M	ROTTWEILLER	F	No lesion	Aortal pseudoaneurisma

N16-6	D	15	POODLE	M	Adenoma	End - stage kidney (Renal failure)
N16-13	D	5	ENGLISH SHEEPDOG	M	No lesion	Bacterial meningitis
N16-16	D	Adu lt	SPRINGER SPANIEL	M	No lesion	Possible enterotoxaemia
N16-18	D	3	LABRADOR RETRIEVER	F	No lesion	Myocarditis
N16-22	D	1	ENGLISH BULL TERRIER	M	No lesion	Euthanasia - Aggression
N16-25	D	4	JACK RUSSELL	F	No lesion	Granulomatous meningoencephalitis
N16-28	D	9	LABRADOR RETRIEVER	F	Cysts	Schwannoma (trigeminal nerve root)
N16-36	D	3	DOGUE DE BORDEAUX	M	No lesion	Astrocytoma
N16-43	D	6	SPRINGER SPANIEL	F	No lesion	Acquired hepatosystemic intra-hepatic shunt
N16-46	D	5	AMERICAN BULLDOG	F	Cysts	Cutaneous mast cell tumours
N16-47	D	8	GERMAN SHEPERD	M	No lesion	Inflammatory bowel disease
N16-48	D	11	JACK RUSSELL	F	No lesion	Plyometric
N16-49	D	8 M	AUSTRALIAN SHEPHERD	M	No lesion	Septicaemia
N16-55	D	3	WEIMARANER	M	No lesion	Septicaemia - skin ulceration
N16-57	D	6	BOXER	F	Cysts	Septicaemia
N16-59	D	5 D	FRENCH BULLDOG	F	No lesion	Cleft palate
N16-64	D	3 M	CAVACHON	M	No lesion	Parvovirus
N16-70	D	6	SHIH TZU	M	No lesion	Congenital porto-systemic shunt + Hepatic encephalopathy
N16-75	D	11	CROSSBREED	M	No lesion	Spinal disc protrusion
N16-82	D	6	LURCHER	M	Hypophysitis	Immunomediated polyarthritis-myocarditis

N16-84	D	3	GREYHOUND	M	No lesion	Haemoabdomen - Renal vein rupture
N16-85	D	10	BOXER	M	Cysts	Meningioma
N16-86	D	11	COCKER SPANIEL	M	Adenoma	Chronic renal failure
N16-96	D	8	GREYHOUND	M	No lesion	Alimentary lymphoma
N16-99	D	1	GREYHOUND	M	Cysts	No diagnosis
N16-101	D	3	CHIHUAHUA	F	Cysts	Bladder ischemia - Post-castration complication
N16-103	D	2	GREYHOUND	M	No lesion	Myocardial and skeletal necrosis
N16-106	D	12	LABRADOR RETRIEVER	F	No lesion	Aspiration pneumonia
N16-107	D	13 W	CHIHUAHUA	F	No lesion	Poliencephalomalacia - Toxic ?
N16-108	D	4	GREYHOUND	F	No lesion	Immunomediated myocarditis
N16-109	D	10	WEST HIGHLAND WHITE T.	F	No lesion	Diabetic nephropathy
N16-112	D	11	STAFFORDSHIRE BULL T.	M	No lesion	Unknown
N16-118	D	6	DOGUE DE BORDEAUX	M	No lesion	Unknown
N16-131	D	6	CHIHUAHUA	M	No lesion	Granulomatous meningoencephalitis
N16-137	D	6	GERMAN SHEPHERD	F	Adenoma	Myocardial degeneration
N16-150	D	10	COCKER SPANIEL	M	No lesion	Spinal disc herniation
N16-151	D	6 W	BORDER TERRIER	M	No lesion	Congenital spinal hypomyelogenesis
N16-152	D	6 W	BORDER TERRIER	M	No lesion	Congenital spinal hypomyelogenesis
N16-154	D	4	STAFFORDSHIRE BULL T.	M	No lesion	Euthanasia - Aggression

N16-155	D	7	BOXER	F	Cysts	Meningitis
N16-156	D	2	GREYHOUND	M	No lesion	Unknown
N16-166	D	7	COCKER SPANIEL	M	Cysts	Aortic stenosis
N16-173	D	12	LABRADOR RETRIEVER	M	Adenoma	Hemangiosarcoma
N16-174	D	4	SCOTTISH DEERHOUND	M	No lesion	Neutrophilic Myocarditis
N16-175	D	2	ROTTWEILLER	F	No lesion	Membranoproliferative glomerulonephritis
N16-177	D	10	BORDER COLLIE	M	Metastasis + Cysts	Sinus adenocarcinoma
N16-178	D	10	SPRINGER SPANIEL	M	No lesion	Unknown
N16-184	D	8	MASTIFF X	F	Adenoma	Glioblastoma
N16-192	D	7	JACK RUSSELL	M	No lesion	Necrotizing meningoencephalitis
N16-194	D	9	COCKER SPANIEL	M	No lesion	Hemangiosarcoma
N16-197	D	11	GREYHOUND	F	Adenoma	Cardiorespiratory failure
N16-207	D	9	GERMAN SHEPERD	M	No lesion	Unknown
N16-210	D	8	CROSSBREED	F	Cysts	Severe acute pancreatitis
N16-215	D	7	GREYHOUND	M	Adenoma	Spinal disc herniation
N16-221	D	5	BORDER TERRIER	M	No lesion	Unknown
N16-223	D	7	CHIHUAHUA	F	No lesion	Astrocytoma
N16-236A	D	2 W	LEONBERGER	F	No lesion	Fading puppy syndrome
N16-236B	D	2 W	LEONBERGER	M	No lesion	Fading puppy syndrome
N16-237	D	9	GOLDEN RETRIEVER	F	Cysts	Septicaemia

N16-238	D	3	SPRINGER SPANIEL	M	No lesion	Intracranial haematoma
N16-239	D	1	BORDER COLLIE	F	No lesion	Unknown
N16-253	D	2	LABRADOR RETRIEVER	F	Cysts	Intestinal lymphoma
N16-255	D	9	STAFFORDSHIR E BULL T.	M	No lesion	Ethylene Glycol intoxication
N16-264	D	8	WEST HIGHLAND WHITE T.	F	No lesion	Unknown
N16-271	D	2	GREYHOUND	F	No lesion	Unknown
N16-285	D	13	DACHSHUND CROSS	M	Adenoma	Trauma
N16-316	D	2	GREYHOUND	F	No lesion	Brachycephalic syndrome
N16-323	D	1	BRITISH BULL DOG	M	No lesion	Unknown
N16-331	D	8 W	LEONBERGER	M	No lesion	Gemystocytic astrocytoma
N16-333	D	6	STAFFORDSHIR E BULL T.	F	No lesion	Polyoencepathopathy - L-2-hydroxyglutaric aciduria
N16-338	D	9	BORDER COLLIE	F	Metastasis	Cerebellar metastasis
N16-342	D	9	CROSSBREED	F	No lesion	Lung oedema
N16-344	D	12 W	SHIH TZU	F	No lesion	End-stage kidney
N16-349	D	14	SPRINGER SPANIEL	M	No lesion	Cerebral infarction
N16-361	D	4	ROTTWEILLER	F	No lesion	Unknown
N16-364	D	5	STAFFORDSHIR E BULL T.	F	No lesion	Glioblastoma
N16-375	D	8	LABRADOR RETRIEVER	F	Hypophysitis	Adrenal atrophy
N16-388	D	8 M	LABRADOR RETRIEVER	F	No lesion	Dilated cardiomyopathy

N16-389	D	8	LEONBERGER	M	Adenoma	Hemangiosarcoma
N16-392	D	7	GREYHOUND	M	Carcinoma	Pituitary carcinoma

Table 2. Signalment of the cases. Id = Identification; D= Dog; C= Cat; m= month; w= week; d= days; F= Female; M= Male; += Neutered/spayed; - = Entire

1.9 Case report: canine hypophysitis

Publication: Hypophysitis, panhypopituitarism and hypothalamitis in a Scottish terrier dog. L. Polledo, M. Oliveira, J. Adamany, P. Graham and K. Baiker. Journal of Internal Veterinary Medicine. J Vet Int Med, 31(5):1527-1532. doi: 10.1111/jvim

1.9.1 Abstract

A 6-year old male neutered Scottish terrier was referred with a one week history of progressive lethargy and anorexia. Neurological examination localized a lesion to the forebrain and hormonal testing showed panhypopituitarism. Magnetic resonance imaging (MRI) of the brain revealed a rounded, well-defined, suprasellar central mass. The mass was slightly hyperintense to the cortical grey matter on T2-weighted (T2W), hypointense on T1-weighted (T1W) images and without T2* signal void. There was a central fusiform enhancement of the mass after contrast administration which raised the suspicion of a pituitary neoplasm. Histopathologic examination revealed a lymphocytic panhypophysitis of unknown origin (suspected autoimmune) involving the hypothalamus (hypothalamitis). This is a unique case report of a dog presenting with inflammatory hypophysitis and hypothalamitis of suspected autoimmune origin with detailed clinical, MRI, histology and immunohistochemistry findings which to our knowledge, have not been previously reported.

1.9.2 Background

Panhypopituitarism refers to decreased secretion of pituitary hormones, which can result from different diseases (non-functional pituitary neoplasia, the local effects of another expanding mass, or secondary to other systemic or regional condition among others) affecting the hypothalamus or the pituitary gland . Primary inflammatory lesions of the pituitary gland are extremely rare in dogs, and in humans, they occur much less commonly than pituitary adenomas. Lymphocytic hypophysitis (LH) is the most common primary hypophyseal inflammatory disorder in humans, and it is speculated to have an autoimmune basis with a focal or diffuse inflammatory infiltration of the pituitary gland and varying degrees of gland destruction. Endocrine symptoms may include partial or total hypopituitarism, with ACTH deficiency being the earliest and most frequent alteration. The hypophyseal form of diabetes insipidus develops as a result of damage of the pars nervosa, infundibular stalk or supraoptic nucleus in the hypothalamus.

Only very few cases of a lymphocytic adenohypophysitis have been reported in dogs so far and none have reported extension of the inflammation to the neurohypophysis and/or hypothalamus along with detailed clinical findings and MRI. In people, inflammatory hypophysitis is a rare but well-described condition known to mimic pituitary neoplasia on computed tomography scans and MRI and its treatment options are promising. Therefore, hypophysitis should be included in the list of differential diagnoses of a presumptive mass located in the hypophyseal or suprasellar area as timely anti-inflammatory management provides a potential treatment option ⁹. This unique case report presents the clinical, radiological and pathological characteristics of this rare condition in dogs.

1.9.3 Case history and clinical findings

A six-year-old Scottish terrier neutered male was referred to the Pride Veterinary Centre (United Kingdom) for progressive lethargy and anorexia of a week duration. On clinical examination, he was lethargic with no other clinical abnormalities detected. The only haematological abnormality detected was a borderline erythrocytosis. Serum biochemical abnormalities included mildly increased creatinine (129 mmol/L; RI 27-124 mmol/L) and mild hyponatremia (135 mmol/L; RI 138-160 g/l). Thoracic radiographs were unremarkable. Abdominal ultrasonography revealed a subjectively small liver with 1-2mm hyperechoic areas with occasional distal shadowing, and liver cytology revealed moderate vacuolar hepatopathy. The dog was hospitalized on isotonic intravenous crystalloid fluids and supplemental feeding through a nasoesophageal feeding tube, but remained depressed. On the following day, the dog showed a low carriage of the head and hind limb ataxia. Neurological examination revealed a decreased nasal sensation bilaterally, but the rest of cranial nerve examination, postural reactions, spinal reflexes and spinal pain were unremarkable.

MRI of the brain was performed with a 1.5T permanent open magnet (General Electric MR; UK). T2-weighted (T2W), T1-weighted (T1W) pre- and post-intravenous contrast administration (0.1 mmol/kg bodyweight of gadopentate dimeglumine [Dotarem; Guerbet, UK]) in transverse and sagittal planes. Fluid attenuated inversion recovery (FLAIR) and Gradient-echo sequences in transverse plane and T1W pre- and post- contrast in dorsal plane were also acquired. Dorsal and slightly rostral to the pituitary fossa there was a rounded well defined central mass measuring about 12mm in diameter. The mass was slightly hyperintense to the cortical grey matter on T2W, hypointense on T1W, without T2* signal void. There was a central fusiform enhancement of the mass after administration of contrast medium. This was best seen with fusion images obtained by summing T2W and T1W contrast images (Fig 12). There was a regional meningeal enhancement ventrally, mostly rostral to the described mass. Both medial retropharyngeal LN were enlarged with heterogeneous enhancement. The suprasellar mass was initially suspected consistent with a neoplasm (macroadenoma or carcinoma and less likely lymphoma) or an inflammatory granuloma.

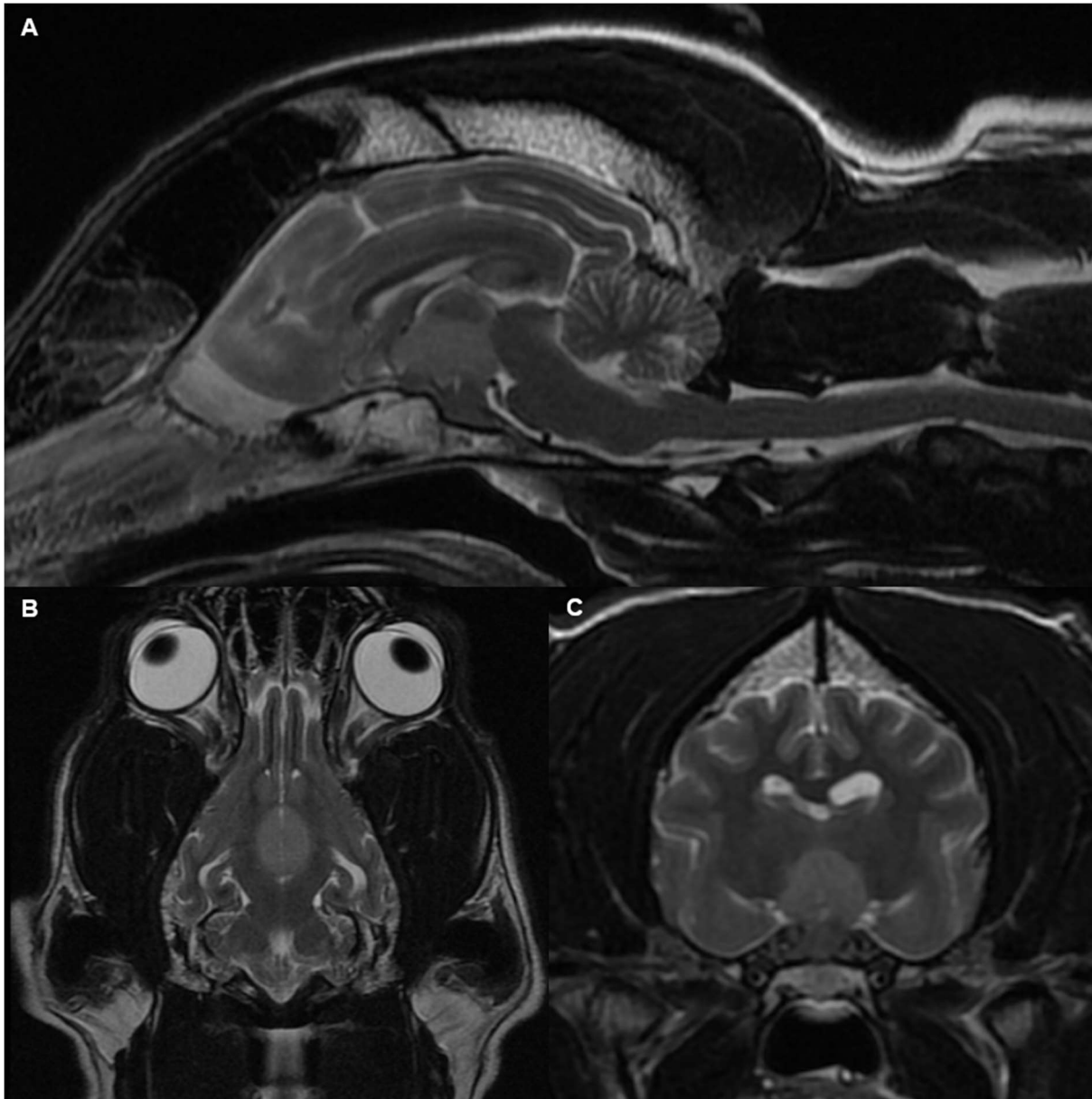


Figure 12 Midsagittal (A), dorsal (B) and transverse (C) T2-weighted images of the brain. There is a well-defined, slightly hyperintense to the normal cortical grey matter central mass in the hypophysis/hypothalamic region.

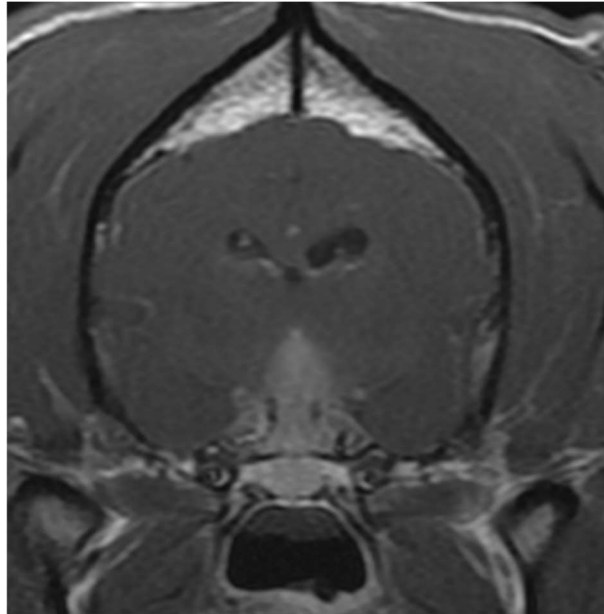


Figure 13 Transverse T1-weighted post contrast image showing central fusiform enhancement of the mass.

Testing of pituitary function was initiated, however, due to an acute deterioration, full testing was not possible. Total Thyroxine (T4) and thyroid stimulating hormone (TSH) were both low at < 5.15 nmol/L (Reference interval (RI): 13.5-50) and < 0.03 ng/mL (RI: 0-0.6 ng/mL), respectively. Secondary hypothyroidism was strongly suspected due to the relatively low TSH and documented pituitary lesion, and supplementation with levothyroxine was initiated at 0.02 mg/kg by mouth once daily. Due to marked deterioration prednisolone at 0.25 mg/kg by mouth once daily was added to treatment while endogenous ACTH and basal cortisol results were pending. Unfortunately, the ACTH stimulation test could not be performed prior to the deterioration of the patient.

Despite the hormonal supplementation, the dog showed a marked deterioration and became stuporous. An increase in body temperature was also noticed (40° C). Blood tests revealed a mild neutrophilic leucocytosis, presence of toxic neutrophils and increased sodium (200 mmol/L; RI: 139-150mmol/L). Urine analysis identified a mild bacterial urinary tract infection. The acute hypernatremia was suspected to be due to a decompensated diabetes insipidus, which was interpreted as most likely due to secondary damage of the neurohypophysis. Exogenous ADH supplementation at 0.5 IU/kg was started and fluid therapy was calculated to gradually decrease the sodium levels. Cefuroxime at 10-15 mg/Kg every 8 hours was introduced to treat the lower urinary tract infection. Although sodium levels decreased gradually, the dog deteriorated very quickly, went into respiratory failure and died.

The endogenous ACTH and basal cortisol results were received after the dog died. Basal cortisol was low <20 nmol/L (RI: 28-250) but endogenous ACTH was not increased at 41 pg/mL (RI: 10-110 pg/mL). The absence of an elevated ACTH in combination with low cortisol was suggestive of secondary hypoadrenocorticism in the absence of pre-sample glucocorticoid therapy.

1.9.4 Post mortem examination and histopathology

The body was submitted to the Veterinary Pathology Service of the University of Nottingham for post-mortem examination. Upon removing the brain, the *sella turcica* appeared empty and no pituitary gland was clearly identifiable. There was no visible mass or mass effect macroscopically. Both adrenal glands showed moderate thinning (atrophy) of the adrenal cortices with a corticomedullary ratio of 1:1 (normal range is 2:1). Examination of thyroid glands revealed bilateral mild to moderate atrophy. The parathyroid glands were of normal size.

Microscopic examination showed a severe lymphocytic panhypophysitis with a marked loss of glandular tissue (Fig 14 and 15). A similar leukocytic infiltrate with a higher proportion of plasma cells and few histiocytes infiltrated diffusely the hypothalamus, affecting paraventricular and supraoptic nuclei and hypothalamic periventricular areas with a focal mild infiltration into the ventral third ventricle. A few periventricular vessels showed an eosinophilic, diffuse expansion of the vascular wall which was negative on Congo red and positive on Periodic Schiff acid stain (plasma leakage, fibrinoid degeneration). Other periventricular vessels showed a variable but mainly lymphoplasmocytic infiltration into the Virchow-Robin space with a multifocal, mild-to-moderate lymphocytic vasculitis. The presence of a small number of intralesional histiocytes rendered a focal granulomatous meningoencephalitis (focal GME) a possible differential diagnosis, even though the lack of angiocentricity and the pituitary involvement would be unusual. Samples of the pituitary gland and hypothalamus were also stained using Giemsa and Gram stains which were negative for bacterial, fungal and protozoal agents.

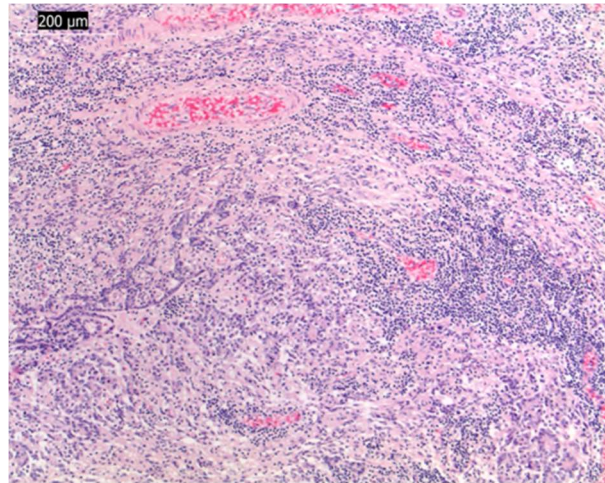


Figure 14 Light micrographs of paraffin embedded section stained with haematoxylin and eosin. Diffuse lymphocytic inflammation of the hypophysis. Bar indicates magnification (x10).

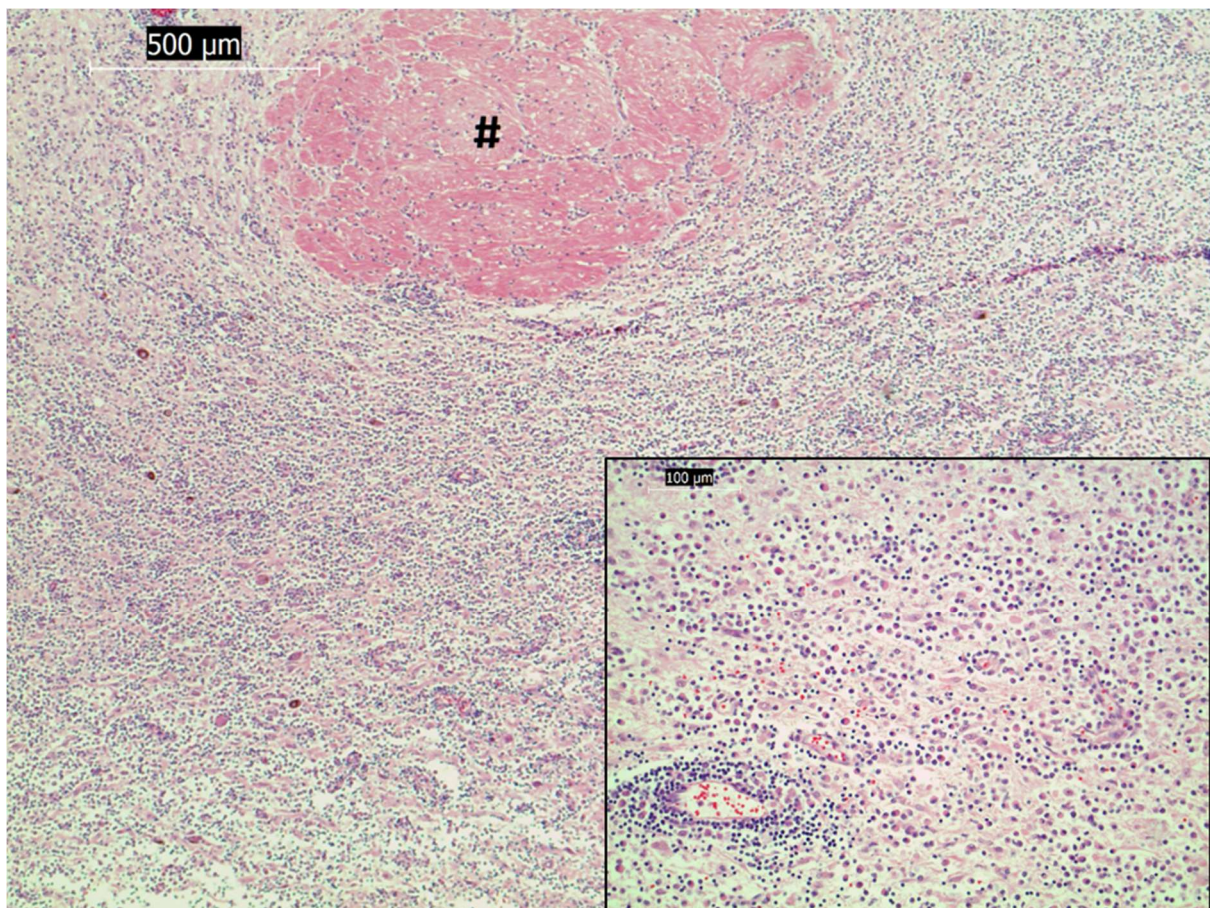


Figure 15 Light micrographs of paraffin sections stained with haematoxylin and eosin. Diffuse lymphoplasmacytic inflammation of the hypothalamus with perivascular cuffing (# shows the non-affected left rostral commissure) (x5 magnification). Inset: higher magnification (x20) of infiltrating leukocytic cell population. Bar indicates magnification.

Immunohistochemistry (IHC) was conducted on tissue sections using antibodies against canine CD3, CD20, Chromogranin A and *Neospora caninum* using appropriate positive and negative controls. IHC of the pituitary gland showed a predominantly T-lymphocytic rich infiltrate with lower numbers of B cells (Fig 16). The inflammation of the hypothalamus showed a higher component of plasma cells (Fig X) and the IHC test for *Neospora caninum* antigen was negative.

The fascicular and the reticular layers of the adrenal cortex from both glands were moderately reduced in thickness (cortical adrenal atrophy) with no signs of inflammation.

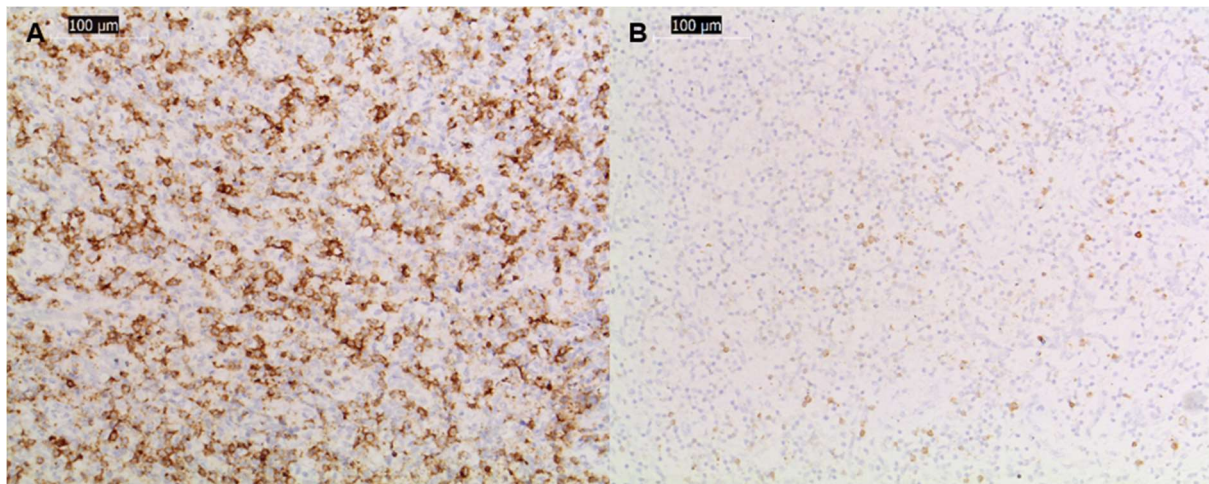


Figure 16 CD3+ (A) and CD20+ (B) immunohistochemical stained serial sections of the inflammatory infiltrate of the hypophysis. Images (A) show high numbers of lymphocyte T (CD3 positive) with less but also abundant B-cells (CD20+ positive). Bar indicates magnification (x20).

1.9.5 Discussion

Histopathological examination of this dog revealed a global infiltration of the pituitary gland extending to the hypothalamus, i.e., lymphocytic panhypophysitis and hypothalamitis. In people, lymphocytic hypophysitis (LH) is a neuroendocrine disorder characterized by an autoimmune inflammation of the pituitary gland with various degrees of pituitary dysfunction, which most commonly affects females in late pregnancy or early post-partum period¹⁵. Cases with a hypothalamic involvement have been reported to be due to antibodies targeting neurohypophyseal hormone-secreting neurons⁴.

After imaging, the case was initially interpreted as a suprasellar mass and the correct diagnosis was made at post-mortem. It is known that MRI findings in people with lymphocytic

hypophysitis are challenging as neither clinical presentation nor MRI findings allow a clear differentiation of hypophysitis from pituitary masses ²⁶. In canine pituitary macroadenomas, MRI findings usually report a relatively well-circumscribed expansile T1W iso- to hypointense and T2W/FLAIR hyperintense mass with strong contrast enhancement. However, pituitary haemorrhage may occur in any pituitary macrotumour, resulting in susceptibility artefacts on T2* images and alteration of signal intensity of the mass on pre- and post- contrast images. In the present case, the pattern of the mass on MRI was unusual for an adenoma, with inhomogeneous central fusiform enhancement, while marked and relatively diffuse enhancement would be expected in a non-necrotic and non-haemorrhagic standard macroadenoma. In people, MRI features more suggestive of autoimmune inflammatory hypophysitis, as compared to pituitary adenoma, include a symmetric enlargement of the pituitary gland, a thickened, non-tapering pituitary stalk and an intact sellar floor and may help to distinguish from pituitary adenoma ^{15,26}. To date, no single radiological sign has proven to have sufficient accuracy in distinguishing with certainty LH from pituitary adenomas.

In human patients, the type, phase and degree of lymphocytic hypophyseal infiltration determine the clinical symptoms. Most frequently, diabetes insipidus, hypopituitarism, headache, and visual defects are reported ^{6,15}. Pituitary insufficiency may affect all pituitary hormones, but normal and elevated plasma levels have also been reported, probably representing different stages of the disease ⁶⁷. In the present case there was reduced TSH and T4, but normal plasma ACTH concentration. Unfortunately, the dog died before the cortisol/ACTH results were obtained. For this reason, an ACTH stimulation test was not performed but the basal cortisol and ACTH were suggestive of a secondary hypoadrenocorticism. Hypothalamic involvement in autoimmune hypophysitis has rarely been described and usually manifests as central diabetes insipidus as seen in our case ^{20,69}. Although previously considered to be due to possible expansion of an inflammatory process from anterior to posterior lobe, with consequent inhibition of the axonal transport of antidiuretic hormone, hypothalamic involvement has now been shown to be due to the anti-hypothalamic antibodies targeting arginine vasopressin secreting neurons, strongly suggesting the presence of hypothalamic autoimmunity in autoimmune hypophysitis ⁴.

In the present case, the etiopathogenesis of the lymphocytic hypophyseal and lymphoplasmacytic hypothalamic inflammation remains uncertain: there was no evidence of an infectious agent. Known idiopathic inflammatory diseases like canine Meningoencephalitides of unknown origin were considered unlikely with the possible exception of a focal GME which is usually more angiocentric and histiocytic, less diffuse within

the neuroparenchyma and when close to the leptomeninges often incites a stronger meningeal inflammation than that observed in this case. To our knowledge, a pituitary involvement has never been described in canine GMEs or in other forms of meningoencephalitis of unknown origin.

The hypophysitis was predominantly lymphocytic with most lymphocytes being CD3 positive T-cells and the adenohypophysis as well as the pars intermedia and neurohypophysis were affected, therefore we suggest an autoimmune, subacute, lymphocytic, panhypophysitis as a likely diagnosis. The increased number of large plasma cells and B-cells in the hypothalamus may represent, as described in the medical literature, an antibody-mediated immune reaction against neurosecretory products like vasopressin. In people, the initial pituitary enlargement in LH, secondary to inflammatory infiltration and oedema, can evolve to progressive diffuse pituitary destruction¹⁵, and this mechanism can explain why the sellar region appeared “empty” on post mortem examination, so-called “empty sella syndrome”. In humans, panhypophysitis affecting the entire pituitary gland is rare but there are some very similar reports to the present case: Two patients with reported necrotizing infundibulo-hypophysitis presented a combination of diabetes insipidus and hypopituitarism and both were suspected to have pituitary tumours with suprasellar extension. Each one was found to have a suprasellar mass lesion with an abnormally thickened enlarged pituitary stalk that intensively enhanced on contrast MRI. However, tissue obtained at transsphenoidal surgery revealed necrosis, fibrosis, and chronic inflammation; there was no evidence of infiltrative, infective, or neoplastic disease processes². These disorders are generally considered idiopathic or autoimmune in origin²⁵. The treatment in these cases consists of replacement of deficient hormones and anti-inflammatory therapy^{14,35}. One case of presumptive LH in a dog resolved following corticosteroid treatment, so both hormonal supplementation and corticosteroids may be considered a promising option when a case is suspected⁶¹.

Following diagnostic investigation, the dog developed high temperature. This was thought to be due to the inflammation and disturbance of neurons in the preoptic area, the temperature regulating centre within the hypothalamus.

In conclusion, the clinical diagnosis of lymphocytic hypophysitis and hypothalamitis in dogs can be challenging as the clinical presentation does not differ from that of more common pituitary masses and MRI imaging findings, like in humans, may not allow differentiation of

LH from other pituitary masses. An inhomogeneous enhancement of a pituitary mass-like lesion may raise the suspicion of an inflammatory process. In these cases, imaging findings should be combined with other diagnostic techniques to reach a final diagnosis. Considering that infectious diseases play an important role in development of hypophysitis, laboratory tests associated with infectious diseases, may be necessary. In humans, transsphenoidal biopsy is performed for conclusive histopathological diagnoses; however, the biopsy procedure may be traumatic and complicated ²⁵. We conclude that lymphocytic hypophysitis is an important differential diagnosis that should be considered when evaluating canine pituitary changes on MRI, because it warrants very different management and treatment and may have a better prognosis.

1.10 References

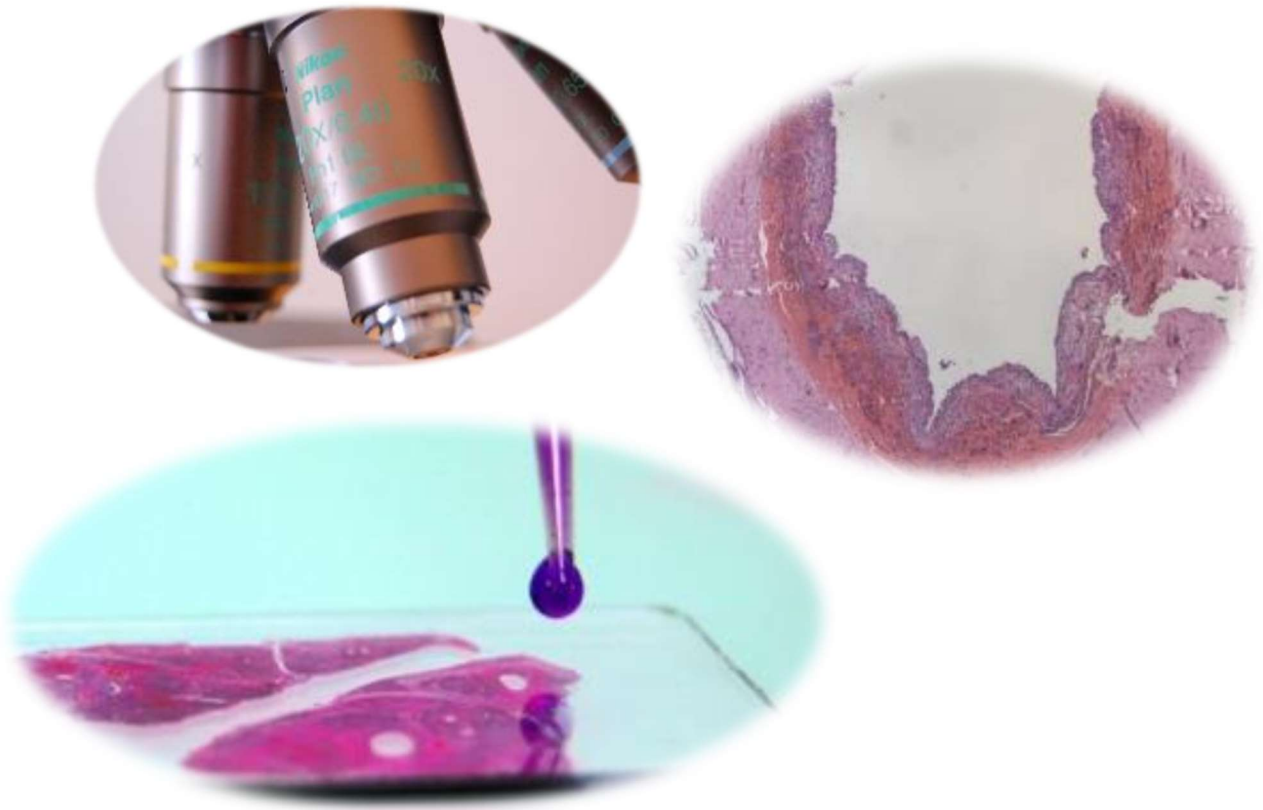
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2 CLINICAL PORTFOLIO



2.1 Introduction

My position was a clinical four-year training program (residency) in Veterinary Anatomic Pathology. Since my start at the School of Veterinary Medicine Science in February 2015 until I passed the certifying examination in February 2018, I had been also officially registered as a trainee (resident) with the European College of Veterinary Pathology (ECVP). The training program at SVMS encompasses diagnostic duties, teaching, seminars and research work. Completion of the program met the eligibility requirements for the Board examinations of the ECVP, which I successfully sat in February 2018.

To achieve the objectives of the program, I worked under supervision within the veterinary pathology service of the University of Nottingham which has been developed around the clinical rotation for final-year veterinary students. With this service, submitting clinicians receive complete diagnostic reports covering the cause of death, gross and histopathology descriptions and diagnoses, pathologist's comments, and imaging as appropriate. I participate in the examination and reporting of these submitted biopsies and necropsies that are also overseen by my supervisors Dr Kerstin Baiker, Dr Llorenç Grau Roma, and Dr Simone de Brot, all Diplomates of the ECVP.

The purpose of this chapter is to describe and demonstrate the clinical and professional achievements and development during the clinical training scholarship.

2.2 Objectives

The main objectives of the training program are:

- Provide broadly-based theoretical and practical experience in veterinary pathology.
- Learning and development of skills in descriptive and diagnostic aspects of anatomic pathology, as well as in the identification and evaluation of pathological processes leading to disease.
- The training is designed to prepare the resident for a career as a diagnostic Veterinary Pathologist and intended to prepare for the ECVF certifying examination.



- This training but more so the Master's program being a part of the Scholarship includes a research project that should lead at least to one publication in the broad field of veterinary pathology in a peer-reviewed journal of international repute. In my case, the aim of the proposed research project is to characterise pituitary changes in dogs and cats with or without neurological and/or endocrine changes.
- The outcome of the clinical training shall include the ability to:
 - Recognise normal anatomy and histology
 - Recognise and describe the gross and histological features of pathological changes (morphological diagnosis)
 - Identify or suggest likely causes of disease (aetiological diagnosis)
 - Perform and report on necropsy and surgical specimens in such a way as to provide pertinent and relevant information to submitting individuals and institutions.
 - Understand the mechanisms involved in disease processes (pathogenesis)

- Understand the principles of established and emerging diagnostic procedures and their applications to pathology
- Think independently and be critical about the interpretation of morphological observations

In order to achieve this, my work was divided into the participation of the clinical diagnostic duty, seminars and research.

2.3 Clinical work

2.3.1 Induction to diagnostic pathology duty

In the beginning of my training, I have attended a number of regular formal and informal training sessions. My supervisors (Dr Kerstin Baiker, Dr Llorenç Grau Roma, and Dr Simone de Brot) have facilitated several sessions with myself to instruct me, including:

- How to perform a necropsy and processing samples
- How to recognise and describe the gross and histological features of pathological changes (morphological diagnosis)
- How to relate lesions to outcome with an aetiological diagnosis
- How to take adequate gross images
- How to elaborate a final histopathological report
- How to focus these activities in training for the certificate of ECVP.

2.3.2 Clinical work in the Pathology Service

The Diagnostic Veterinary Pathology Service of the University of Nottingham involves the examination of necropsy and biopsy submission from our Clinical Associates and external referrals in order to obtain an accurate final diagnosis. During this process, I perform small-group practical teaching with 5th year students on their clinical rotation for the two weeks of hands-on training in veterinary pathology.

My duty rota was a two week rolling rotation in:

- **First week:** I am involved in necropsy cases.

- **Second week:** I am involved in biopsy cases.

2.3.3 Case load (2015 – 2018)

- Necropsies:
 - poultry cases: 32 cases
 - bovine cases: 3 cases
 - feline cases: 145 cases

- canine cases: 274 cases
- equine cases: 47 cases
- rodents, among them rabbits: 43 cases
- ovine cases: 15 cases
- ferret case: 3 case
- fish case: 1 case
- Biopsies:
 - poultry cases: 102 cases
 - bovine cases: 5 cases
 - feline cases: 4 cases
 - canine cases: 30 cases
 - equine cases: 3 cases
 - porcine cases: 3 cases
 - ovine cases: 3 cases

To increase my exposure to biopsy work, I did 2 placements (one-week duration each) at the Royal Veterinary Collage of London (UK), where I was fully involved in the Diagnostic service performing a total of **75 small animal (mainly dogs and cats) biopsies**.

2.3.4 Description of the necropsy and biopsy work

The Diagnostic Veterinary Pathology Service of the University of Nottingham receives every week between 10-20 bodies for diagnostic purposes from different species and clients. The following steps explain my role for each case during my week of necropsy duty with the 5th year students on rotation.

- **Presentation of the case and its clinical history to the students (15 minutes):**

Before starting the necropsy, the students are given at least 10 minutes to think and discuss among them the clinical history. Then, I join them and we discuss altogether their thoughts, differential diagnoses, and sampling. Also we define the role of each student performing the necropsy (writing the reports, taking pictures, cutting, etc.).

- **Necropsy procedure and supervision of the students while they are carrying out the necropsy (2-4 hours)**

The 5th-year students are practically performing the dissection of the body - after some first day training -under by my full-time supervision. This implies:

- Close supervision of the necropsy procedure
- Discussion of every pathological or artificial lesion shown in the organs
- Coordination of duties done by the students
- Photography work
- Preparation of all the organs to the senior pathologist who supervised the case for the final discussion.
- Sampling: Small 1 x 1 x 1 cm samples are systematically collected from every organ in 10% formalin.
- Phone call to give some preliminary information to the clinician.

For a few weeks per year, the Pathology service does not have undergraduate students on rotation and the full post mortem examination remains in my responsibility. Tissue sampling of all relevant body organs including potential samples for additional testing for toxicological analysis or microbiology (15-1h minutes each case)

Routine tissue samples are stored in formalin at least for 24-48h. Afterwards, I am in charge of trimming in samples for histology which consists in:

- Selection and sampling of the relevant affected areas
- Trimming the selected samples in max 0.5 cm slices and labelling of the corresponding cassettes
- Placement of the samples in histologic cassettes in 10% formalin.
- Prepare them to be collected by the histology technician.

- **Examination of the histology samples and writing the draft of the final report (variable depending on the case ~ 1-6 hours)**

Students were in charge of recording the macroscopic findings and writing the description and interpretation of the gross findings, which I had to review, correct and to complete with photodocumentation.

Once the histology slides were received from the lab, I had to carry out a microscopic examination and to write a detailed full description of the histopathological findings to elaborate a draft report that was sent to the supervisor in charge of the case. Once revised, the final report was sent to the corresponding clinician.

- **Description of the biopsy work**

- Taking adequate gross pictures of each sample received.
- Trimming of the cases
- Elaborate a preliminary gross report.
- A microscopic study of each sample and the description of the finding to also carry out a histopathological report.

- **Examples of these reports:**

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PATHOLOGY REPORT

Species: Canine	Breed: Labrador	Pathology Lab No: N18-100
Age: 2 years 0 months	Sex: Male Neutered	Pathologist:
Owner:		Date of death:
Animal name/ID:		Date of necropsy: 01/03/18
Body weight: 37.85Kg		Euthanasia? Yes
Referring Veterinary Surgeon:		
Clinical Features/history:		
Icterus, acute kidney injury, thrombocytopenia, ulcerative skin and mucosal lesions.		
Admitted on Saturday 24/02/18 with worsening signs of acute kidney injury and jaundice over the		

(Information in green box above as provided on submission form.)

Gross description:

Examined is a male neutered Labrador in good body condition (score BCS= 4/5) with normal subcutaneous and abdominal fat stores.

A watery red-tinged fluid can be seen at both nares and a moderate amount of thick red fluid can be seen in the mouth with multifocal blood clots on the oral mucosa.

Mucous membranes and hairless regions including the internal pinnae are diffusely pale and yellow (icterus).

Multifocal, well demarcated, flat, circular red lesions up to 1cm in diameter (ecchymosis) are present on the ventral abdomen and prepuce. Dark tarry faeces can be seen at the anus.

Extending from the interdigital space toward the hock, on the lateral aspect of the left hind limb there is a 9 x 5 cm focally extensive and reddened loss of epidermis (severe ulceration,

Figure 1). Approximately 10 cm in diameter, there is a large, focal well demarcated, raised red gelatinous mass (haematoma) subcutaneously on the left costal arch.

On opening of the abdomen and thorax, there are multifocal to coalescing flat, well demarcated red circular lesions up to 2 mm in diameter (petechiae and ecchymosis) throughout the mesentery, intestinal serosa and mucosa, serosa of the abdominal cavity and coronary sulcus of the heart.

Subcutaneous and abdominal fat is diffusely pale yellow (icterus).

Fifty ml of red, watery, serosanguinous fluid is collected from the thorax (mild to moderate hydrothorax). A red, foamy fluid is seen in the distal trachea (pulmonary oedema). Affecting 80% of the lungs, there are multifocal to coalescing dark red areas (congestion) with no change in consistency (Figure 2). Multifocal to coalescing, red, well demarcated areas, are seen in the left cranioventral lobe (haemorrhages), which also sunk when placed into formalin (consolidation). 25 ml of watery red fluid is collected from the pericardial sac (mild to moderate hydropericardium).

The liver is diffusely pale with an orange tinge (icterus) and an accentuated diffuse lobular pattern (Figure 3). Two well demarcated, circular, flat, dark- red discolourations (suspected infarcts) can be seen within the gall bladder (Figure 4).

The stomach contains dark brown ingesta and there are thick, very dark black/green fluid contents within the colon. Multifocal, well demarcated white, regular, circular up to 2mm in diameter can be seen in the caecum from the serosa areas (mucosal associated lymphoid hyperplasia). The mesenteric lymph nodes are diffusely dark but normal size (probably associated with multiple petechiae and ecchymosis).

There is mild to moderate splenomegaly (25cm in length).

The kidneys are diffusely swollen and pale-yellowish (Figure 5) and there are multifocal to coalescing red dots up to 1mm in diameter (petechiae) on the surface of the kidney and within the cortex. The bladder mucosa is diffusely pale and yellow (icterus) with multiple, dense free floating red clumps (blood clots) within the lumen.

Gross diagnoses:

- **Severe icterus**

- **Cutaneous:** Severe, acute, focally-extensive haemorrhagic ulceration to the lateral aspect of the left distal hind limb.
- **Kidneys:** Moderate, acute, diffusely swollen and pale kidneys with petechial haemorrhages in the cortex (suspected acute kidney injury).
- **Oral mucosa, abdomen and prepuce, mesentery, intestinal mucosa, abdominal serosa, renal cortex, mucosa of urinary bladder and coronary sulcus:** Numerous, acute multifocal petechial and ecchymotic haemorrhages.
- **Lung:** Multifocal, severe, craneoventral lung consolidation with haemorrhages and diffuse pulmonary oedema and congestion
- **Heart:** Mild to moderate hydropericardium
- **Thoracic cavity:** Mild to moderate hydrothorax
- **Gall bladder:** Two focal, acute, severe, infarctions
- **Intestine:** Severe haemorrhagic diarrhoea

Histopathology:

Skin: There is a focally-extensive loss of the epidermis (ulceration). The superficial dermis is diffuse covered by a marked leukocytic infiltrate of mainly neutrophils (mostly degenerated) admixed with fibrin and haemorrhage. The subjacent dermis presents oedema, multifocal haemorrhages, and multifocal to coalescing extensive areas of coagulative and lytic necrosis mainly affecting the adnexa with a moderate multifocal neutrophilic infiltrate (suspected area of infarction). Multiple dermal arterioles show fibrinoid necrosis of the arterial wall, which appears often disrupted and/or lost; and is also infiltrated by degenerated neutrophils with abundant leukocytic debris (marked leukocytoclastic vasculitis). There are abundant perivascular haemorrhages.

Kidney: Approximately 30% of the glomeruli are affected by one or more of the following changes that range from mild to severe and segmental to global: partial lytic necrosis or loss of the glomerular tuft, mesangial hypercellularity with presence of neutrophils (mostly degenerated), hypertrophy of parietal cells, and accumulation of proteinaceous material and/or occasional haemorrhage within Bowman's space. Frequently, there is lytic necrosis and presence of karyorrhectic debris within in the area of the *macula densa* (suspected fibrinoid necrosis affecting afferent and efferent glomerular arterioles). There are numerous,

multifocal, foci of acute tubulorrhexis and tubular necrosis with evidence of proteinuria, which appear especially prominent related to areas of haemorrhage, often together with features of early tubular regeneration. Some small vessels show fibrinoid necrosis and are occluded by eosinophilic, hyalinised, material, intermingled with low numbers of degenerate and viable neutrophils (suspected thrombi). Multifocally, some tubules contain intraluminal calcium deposits (incidental finding). There is minimal leukocytic interstitial inflammation. Warthin-Starry or Gram stains did not reveal any organisms (such as Leptospire) within the tissue sections.

Lung: There are multifocally to coalescing, extensive haemorrhages, and moderate to marked accumulation of fluid within alveolar spaces (pulmonary oedema) admixed with foamy macrophages. Within the cranial lung lobes, there are multifocal inflammatory foci containing numerous viable and degenerated leukocytes with streaming, lightly basophilic, homogeneous chromatin occupying the lumen of mainly bronchioles but also alveoli. This infiltrate often appears infiltrating and causing rupture of the bronchiolar wall, and is mostly related to the presence of eosinophilic, acellular, up to 20 µm and translucent particles (some look like plant debris) (suspected foreign body – aspiration pneumonia?). Upon staining with gram stain, numerous gram +, coccoid-shape, up to 3 µm, bacteria are observed. Multifocally, there are vessels presenting fibrinoid necrotizing vasculitis with occasional presence of attached fibrin thrombi with embedded degenerated neutrophils.

Liver: Most of the portal and centrilobular vessels show necrotizing vasculitis, as described above. There is moderate dissociation of the hepatic cords. Sinusoidal spaces are widened by diffuse moderate congestion. Some bile plugs are seen (cholestasis). There are moderate numbers of haemosiderin-laden Kupffer cells and hepatocytes.

Intestine: The mucosa is diffusely autolytic. There are moderate numbers of multifocal haemorrhages within the submucosa and serosa.

Histopathological diagnoses:

- **Blood vessels (skin, kidney, lung and liver):** Severe, multifocal, acute, neutrophilic leukocytoclastic and necrotizing vasculitis with occasional fibrin thrombi (septic)

- **Skin:** Focally-extensive, severe, subacute, dermal and epidermal infarction with ulceration with multifocal haemorrhage and oedema
- **Kidney:** Moderate, focal, acute, necrotizing glomerulitis, with multifocal, moderate, acute, tubular necrosis with proteinuria and multifocal haemorrhages.
- **Lung:** Moderate, unilateral (left), cranioventral, multifocal to coalescing, acute, neutrophilic and necrotizing pneumonia with presence of foreign body material (aspiration pneumonia?), abundant coccoid bacteria (suspected streptococci/staphylococci), and multifocal severe haemorrhages and pulmonary oedema.
- **Liver:** Moderate hepatic cord dissociation with moderate, diffuse, sinusoidal congestion, and mild cholestasis.

Comment:

This dog died very likely due to the severe renal failure and vasculopathy (including necrotizing vasculitis, thrombosis and very likely DIC) but aetiology is not clear. Pulmonary lesions appear to be bacterial in origin, however whether this bacterial proliferation is primary or secondary is not known. Icterus is very likely pre-hepatic in origin.

Main differential diagnoses include: streptococcal toxic-syndrome, leptospirosis, cutaneous and renal glomerular vasculopathy, haemolytic uraemic syndrome (HUS) and any other acute septicemic agent causing disseminated intravascular coagulation (DIC).

Streptococcus spp (such as *S. equi sub. zooepidemicus*) are opportunistic pathogens that can cause localized infection (including pneumonia) and septicemia in dogs. It has occasionally been related with toxic shock-like syndrome, which resembles the toxic shock syndrome in people, and is characterized in dogs by painful, deep dermal or subcutaneous lesions along the limbs or trunk, accompanied by severe hypotensive shock and disseminated intravascular coagulation with infarctions and multiorgan affection (*Streptococcal Infection in Dogs, Lamm et al 2010*). In this case, a possible initial aspiration pneumonia (river water?) might have facilitated the streptococcal invasion and further development of toxic syndrome-like lesion.

The sensitivity of Warthin-starry stain for *Leptospira* detection is not high so further testing (e. g. immunohistochemistry or PCR) needs to be performed to rule out this possibility.

Cases with lesions in the skin, lung and kidney similar to the ones in the present case have been previously diagnosed as 'idiopathic cutaneous and glomerular vasculopathy' firstly in the US (so-called Alabama Rot), and also more recently in the UK (so-called New forest disease). Despite several investigations, the cause of it is still unknown, and an E. coli role in development of this disease has been suggested. Furthermore, it is unknown at this time whether canine HUS and CRGV are truly two distinct disease processes (*Cutaneous and renal glomerular vasculopathy as a cause of acute kidney injury in dogs in the UK. Holm et al. 2015*).

Tissue from liver, kidney, urine and stomach contents have been collected and stored frozen (for the following 2 months) in case further testing is required.

Pictures:



Figure 1 Cutaneous ulceration



Figure 2 Congestion of the lungs with multifocal to coalescing haemorrhages in craneoventral lobes (yellow circle)

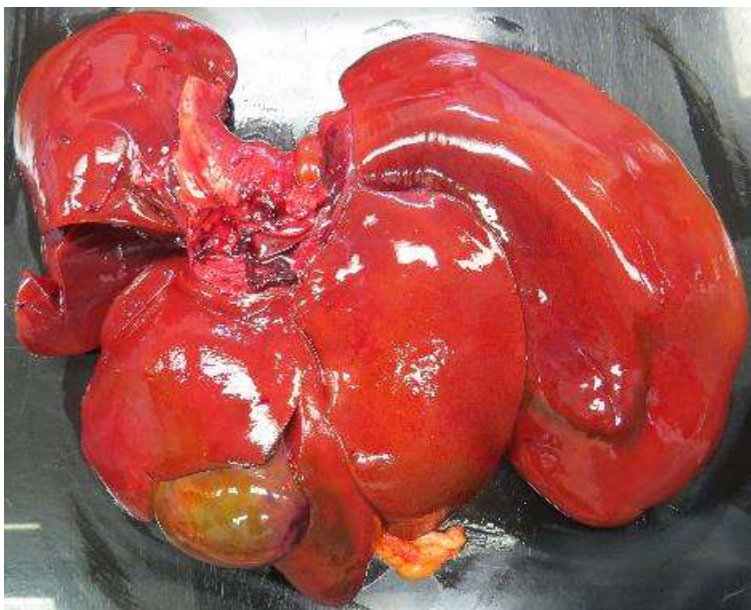


Figure 3 Liver: Icterus



Figure 4 Gall bladder infarcts

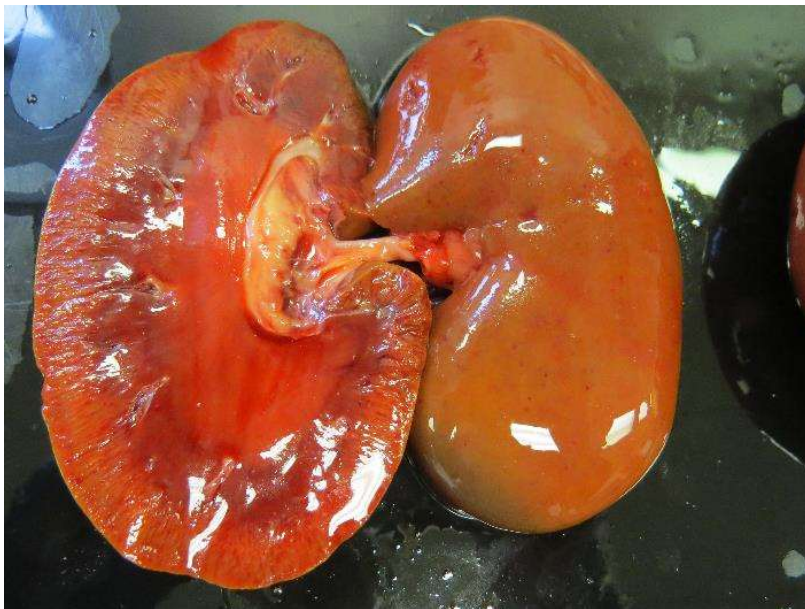


Figure 5 Pale kidneys with multifocal petechiae

BIOPSY REPORT

OPST REPORT

Species: Canine	Breed: German Wire-haired pointer	Pathology No: B16-114
Age: 5 years	Sex: Female	Pathologist:
Owner:		Date received: 22/06/2016
Animal name/ID: Trio		Date completed:
Referring Veterinary Surgeon:		
Clinical Features/history*: Clinical history: Progressive R hemiparesis suggestive of meningioma		

*Information provided above in green box taken from submission form

Samples provided:

C6 intradural – extradural mass

Gross description:

Provided is a 1.6 x 1.1 x 0.9 cm, irregular, white tan, non-encapsulated, meaty in consistency mass. Two whole cross sections are trimmed.

Microscopic description:

Extending to the cut borders, there is a rather homogenous, highly cellular, non-encapsulated, neoplastic proliferation of polygonal cells, supported by moderately dense fibrovascular stroma. Within one tissue section, dense fibrous tissue (suspected dura mater) partially surrounds the neoplastic growth with suspected infiltration of tumour cells. Neoplastic cells form small nests, separated by fine fibrovascular tissue. The cells show distinct cell borders, have moderate to large amounts of pale eosinophilic spongy cytoplasm with a regular round eccentric nucleus with one typically small nucleolus and fine chromatin structure. Anisocytosis and anisokaryosis is mild. Mitotic figures are not observed in 10 40x HPF. Multifocally, areas of moderate necrosis are observed (possibly related to surgical procedure).

Diagnoses:

- Meningioma (meningothelial)

Comment:

Intraspinal meningiomas in dogs are most common in the cervical spinal cord but can be found throughout the neuraxis. Location is correlated with histologic grade, with grade I tumours more likely to be in the cervical region

than grade II tumors ('Canine intraspinal meningiomas: imaging features, histopathologic classification, and long-term outcome in 34 dogs.' Petersen et al. 2008).

Given the histologic pattern, low cellular pleomorphism and low mitotic index support a benign growth (grade I) in this case.

Pictures

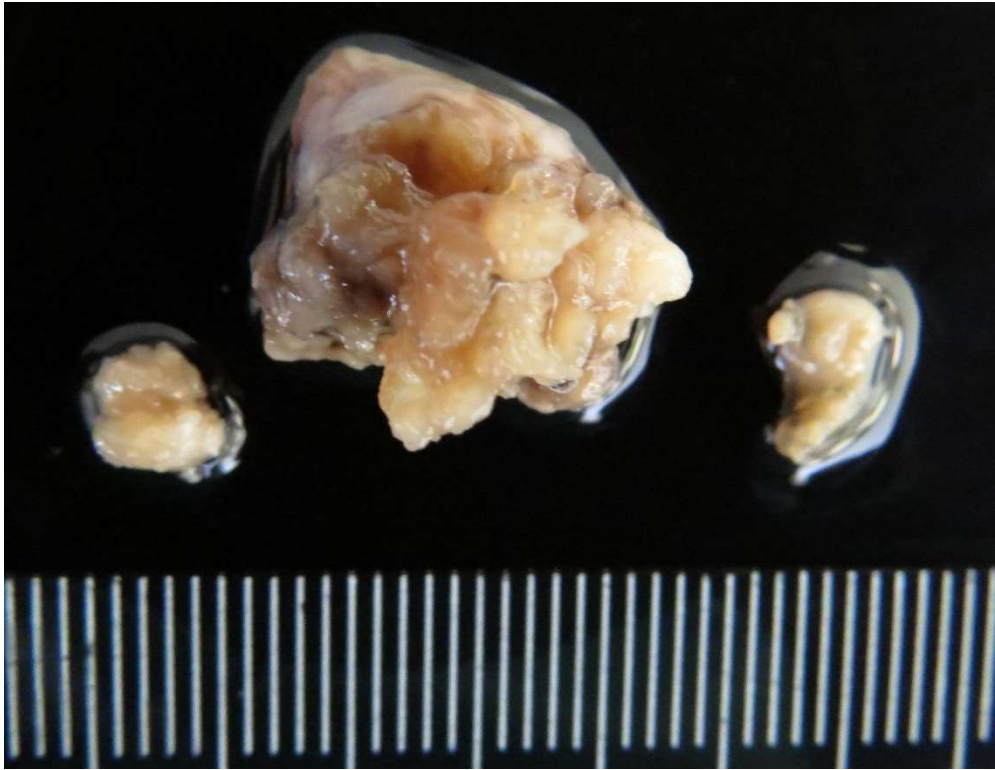


Figure 1

2.4 Rounds and pathology training

I have continued attending seminars and discussion sessions organised by the pathologist team, such as:

- **Histopathological rounds (weekly):** Once a week, one of the supervisors prepares a histopathological slide session. These sessions consist of the description of 2-3 slides following strictly the instructions given by the ECVP, for preparing the histopathological part of the ECVP Board examination. These rounds give me the opportunity of learning how to describe in a systematically, accurate and detailed way.
- **Gross rounds (weekly):** Once a week, I participated in gross image rounds organised by one of the supervisors, often together with other post-graduate students. These rounds consist on practising the gross mock exam from ECVP Board examination, to learn how to reach a complete and precise gross morphologic diagnosis, often relating it to specific aetiologies or pathogeneses.
- **General Pathology (every 3 weeks):** A chapter of the relevant books of the reading list of the ECVP board exam is announced, revised by us postgraduate students within these 3 weeks and tested via a multiple choice quiz.
- **Histopathological Wednesday slide conference discussions (monthly):** I have enjoyed contributing to the histopathological discussions using the training tool 'Wednesday slide conference'. We receive slides which have been previously discussed by the Department of Defence Veterinary Pathology Residency Program (DODVPRP) who trains Army veterinarians in the specialty of anatomic veterinary pathology in the United States.

2.5 Conferences, seminars and CPD attended

- **Nottingham Spring Slide meeting (2015, 2016, Nottingham):** This is a meeting, where pathologists from different Universities of United Kingdom share interesting cases to be discuss. I participated in this meeting with a case of Avian Adenovirus in a turkey (2015) and the case of canine hypophysitis (2016).
- **ECVP Summer School (2015, Bologna, Italy):** I have attended to a 2 weeks-intensive course in Veterinary Pathology from Monday 6th July to Friday 17th July 2015.
- **ECVP Summer School (2016, Berlin, Germany):** I have attended to a 2 weeks-intensive course in Veterinary Pathology in Berlin from Monday 18th July to Friday 29th July 2016.
- **2016 Davis-Thompson Foundation Descriptive Veterinary Pathology Course (2016, Royal Veterinary collage of London, United Kingdom):** I have attended to a 1 week course in Veterinary Pathology from Monday 1st August to 5th August.
- **ECVP Summer School (2017, Berlin, Germany):** I have attended to a 2 weeks-intensive course in Veterinary Pathology in Berlin from Monday 17th July to Friday 28th July 2017.
- **28th meeting of the ECVP (2017, Lyon, France):** I presented in this meeting the results from the pituitary gland project.
- **CL Davis foundation (2017, Lyon, France):** Lymphoma: Update course

2.6 Publications derived from clinical work:

- **Meningeal carcinomatosis and spinal cord infiltration caused by an invasive pulmonary adenocarcinoma in a cat.** Posporis C, Travetti O, Grau-Roma L, Oliveiro M, Polledo L, Wessmann A. J Small Anim Prac. JFMS 23;3(2)
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