Veterinary Pathology

Pathological findings in the pituitary glands of 201 dogs and cats and literature review

Journal:	*Veterinary Pathology*
Manuscript ID	VET-17-FLM-0283.R2
Manuscript Type:	Full Length Manuscript
Date Submitted by the Author:	03-May-2018
Complete List of Authors:	Polledo, Laura; University of Nottingham School of Veterinary Medicine and Science Grinwis, G.C.M.; Utrecht University, Pathobiology Graham, Peter; University of Nottingham School of Veterinary Medicine and Science Dunning, Mark; University of Nottingham School of Veterinary Medicine and Science Baiker, Kerstin; University of Nottingham School of Veterinary Medicine and Science
Keywords:	pituitary adenoma, pituitary carcinoma, Crooke's change, incidentaloma, hyperplasia, pituitary cyst, hypophysitis
Abstract:	With the exception of classic functional adenomas in dogs and horses, pituitary lesions are infrequently described in the veterinary literature. 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 stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS), Gordon and Sweet's reticulin and immunohistochemistry (against ACTH, GH, MSH-α and prolactin) stains for lesion characterisation.Pituitary abnormalities were identified in 36/136 (26.4%) dogs and 10/65 (15.3%) cats. Cystic changes were the most common 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 enabled staining of secretory granules in ACTH-immunoreactive adenomas and reticulin stain helped differentiate them from hyperplastic nodules: adenomas showed PAS positive intracytoplasmic granules and loss of the normal reticulin network. One dog had a pituitary carcinoma with infiltration into the thalamus. Other pituitary abnormalities included: secondary metastases (2 dogs), hypophysitis (4 dogs, 1 cat). In the majority of cases the lesion appeared to be subclinical and could be considered incidental; of those cases with pituitary lesions, clinical manifestations were apparent in 4 dogs (2.9%) and no cats antemortem. Pituitary abnormalities are common in dogs and cats and their wider clinical relevance requires further investigation.

Pathological findings in the pituitary glands of dogs and cats

L. Polledo[1], G. Grinwis[2], P. Graham[1], M. Dunning[1], K. Baiker[1]

[1]School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington, United Kingdom

[2]Department of Pathobiology, Faculty of Veterinary Medicine, Utrecht, The Netherlands

Corresponding author:

Kerstin Baiker, School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington Campus, LE12 5RD, United Kingdom.

Email: Kerstin.Baiker@nottingham.ac.uk

[1]

[1] This manuscript has been prepared in the Uniform Requirements format.

ABSTRACT

With the exception of classic functional adenomas in dogs and horses, pituitary lesions are infrequently described in the veterinary literature. 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. Lesions were characterized in sections stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS), Gordon and Sweet's and reticulin stains, and by immunohistochemistry for ACTH, GH, MSH-α and prolactin. Pituitary abnormalities were identified in 36/136 (26.4%) dogs and 10/65 (15.3%) cats. Cystic changes were the most common lesion, occurring in 18 (13.2%) dogs and 8 (12.3%) cats. Pituitary neoplasia was detected in 14.1% (12/85) of middle-aged and old dogs; 1 (1.5%) cat had pituitary nodular hyperplasia. PAS and reticulin stains helped differentiate ACTH-immunoreactive adenomas from hyperplastic nodules: adenomas contained PAS-positive intracytoplasmic granules and loss of the normal reticulin network. One dog had a pituitary carcinoma with infiltration into the thalamus. Other pituitary abnormalities included secondary metastases (2 dogs) and hypophysitis (4 dogs, 1 cat). In the majority of cases the lesion appeared to be subclinical and could be considered incidental, whereas clinical manifestations were apparent in only 4 dogs (2.9%) and none of the cats with pituitary lesions. Pituitary abnormalities are

common in dogs and cats and their clinical relevance requires further investigation.

KEYWORDS:

Dogs, cats, adenohypophysis, hyperadrenocorticism, Crooke's change, incidentaloma, hyperplasia, pituitary gland, neoplasms, cysts, inflammation

A wide range of abnormalities can occur within the pituitary gland and may lead to various endocrinological or neurological signs. 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 through transsphenoidal hypophysectomy in dogs and cats [22,34,41]. At present, there are limited data in the veterinary literature on acquired pituitary abnormalities, and the data that are available is complicated by lack of consensus over terminology and classification.

In dogs, the incidence of Cushing's disease (pituitary dependent hyperadrenocorticism) is estimated at 1-2 cases per 1000 dogs per year [34], and pituitary adenomas account for 25% of all intracranial neoplasms in middle-age or geriatric dogs [18,43]. In cats, pituitary tumors are considered uncommon, accounting for 9.3 per cent of all intracranial tumors [44], often related to hypersomatotropism leading to insulin-resistant diabetes mellitus [17]. No data currently exist on the presence of silent pituitary lesions (so-called incidentalomas in digital imaging) in dogs or cats to contrast with that in humans. This is important given the increasing use of advanced neuroimaging in clinical practice.

Pituitary lesions can be subclinical or cause endocrine dysfunction (hypofunction or hyperfunction) or neurological signs due to local effects of an expanding mass [37,46]. In humans, approximately 14% of pituitary glands examined at autopsy contain small pituitary adenomas, mainly prolactin-producing, with the vast majority being clinically silent[7,19]. Other pathologic entities such as pituitary cysts and metastatic tumors are thought to have a similar prevalence in humans [25,26].

The aim of the study was to determine the prevalence of pituitary abnormalities in a general population of dogs and cats and to compare it to that seen in humans. In addition, a further aim was to characterize the histopathological features of these abnormalities to help with understanding the range of pituitary lesions encountered in dogs and cats.

MATERIAL AND METHODS

Cases and histopathology

A total of 201 pituitary glands were randomly 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 (interquartile range: 2 - 8.5 y; range: 4 days to 14 y) with age distribution as follows: 51 young (<4 y), 43 middle-aged (4-<8 y) and 42 old-aged (≥8 y) dogs. Cats had a median age of 9 y (interquartile range: 4 - 14 y; range: 1 month to 20 y), with age distribution as follows: 14 young (<4 y); 12 middle-aged (4-<8 y) and 39 old-aged (≥8 y). The proportion of males was 57% of the dogs and 55% of the cats. The neuter status for both sexes was 42% and 72% of the dogs and cats respectively. The most common canine breeds represented 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, sex, breed, clinical history and final diagnosis were recorded from each animal (Supplemental Table S 1).

Pituitary glands were immediately fixed in 10% neutral-buffered formalin before performing a sagittal section of each gland and both halves were embedded

in paraffin wax. Tissue sections (5 µm) were stained with hematoxylin and eosin (HE) for routine microscopic analysis. Measurement of the size of the lesions was performed on the histologic section with the widest lesion diameter (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.

Special stains and immunohistochemistry (IHC)

Tissue sections of thirteen (12 dogs and 1 cat) pituitary glands presenting proliferative changes and one normal control pituitary gland were stained with periodic acid Schiff (PAS) and Gordon and Sweet's reticulin stain using appropriate control tissues for validation of the stains. The same samples were immunolabelled for adrenocorticotropic hormone (ACTH). Three canine samples with neoplastic changes which were entire or partially non-immunoreactive for ACTH were further immunolabelled for growth hormone (GH), alpha melanocyte stimulating hormone (α-MSH) and prolactin. 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, Glostrop, Denmark; and prolactin, noncommercial 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-µm-thick pituitary sections following previously published protocols [3,35].

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.

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 had 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 1. All the cats presenting pituitary cysts were older than 8 y.

Neoplastic and hyperplastic lesions.

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 sexes 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 1 cat, a 23-year-old domestic short hair, had primary pituitary hyperplasia.

Pituitary adenoma. Of the 11 dogs with pituitary adenoma, only 1 (a Dachshund crossbreed) had been diagnosed with hyperadrenocorticism ante mortem, and had typical associated skin and hepatic lesions. Adrenal gland tissue was available from this dog and three others of the 11 dogs with pituitary adenoma, and all 4 histologically presented moderate bilateral adrenocortical hyperplasia. The other 7 (63.6%) dogs had no lesions compatible with hyperadrenocorticism. In a further 33 dogs with no pituitary proliferative change, 10 (30%) dogs also had bilateral adrenal cortical hyperplasia.

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 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. 1). The adenoma in the dog with hyperadrenocorticism was 1.8 mm in diameter. Microscopically, all adenomas were located in the pars distalis and consisted of variably demarcated, densely cellular nodules of neoplastic cells (Fig. 2 a), arranged in sheets (diffuse pattern) with fine supportive stroma, and loss of the normal stromal network pattern as shown by the reticulin stain (Fig. 2 b). Neoplastic cells had little pleomorphism with small to medium-sized nuclei, coarse chromatin pattern, and very characteristic abundant, uniformly light eosinophilic cytoplasm with HE stain (Fig. 2 a), that contained PAS positive granules (Fig. 2 c), and with consistent ACTH immunoreactivity in those cases examined by IHC (Fig. 2 d). No mitotic figures or areas of necrosis were observed in any case. In addition to the described neoplastic cells, the two largest corticotroph adenomas contained cells that did not contain PAS positive cytoplasmic

granules; were not immunoreactive to ACTH, MSH, GH or PRL; had round to oval nuclei with coarse chromatin, and were supported by fine fibrovascular stroma.

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

Pituitary carcinoma. A 7-year-old male greyhound had a pituitary carcinoma manifesting neurological signs, neck pain and low head carriage without signs of an endocrinopathy. On postmortem examination, a 2 x 3 x 2 cm, non-encapsulated mass arose from the pars distalis, and markedly invaded 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 (Fig. 3a and 3b), separated by moderate amounts of fibrous stroma (Fig. S1), and infiltrated the moderately gliotic neurohypophysis and diencephalon (Fig. S1). 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 reaction (Fig. 3 c) and ACTH, MSH, GH, and PRL-IHC were negative (Fig. 3 d).

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-diameter, focal, non-encapsulated, nodular proliferation of secretory cells which caused enlargement of the acini, mildly compressing the adjacent pars distalis, with minimal to no visible

changes in the normal pituitary stromal network in the reticulin stain (Fig. S2). Hyperplastic cells revealed a chromophobic cytoplasm, often vacuolated and poorly stained with PAS, with negative or very weak ACTH immunolabelling.

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 had prominent neurological signs with no signs of an associated endocrinopathy.

Pituitary cysts and cyst-like structures

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, had 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 system [42] (Fig. S3-S6): cysts with epithelial lining were present in 10 dogs and 4 cats; cystic areas without an epithelial lining were present in 7 dogs and no cat (Fig. 5); and enlarged colloid follicles of the pars intermedia were present in 1 dog and 4 cats. The epithelial lining was a cuboidal, ciliated, simple epithelium in 3 dogs, and a non-ciliated, flat to cuboidal simple epithelium in the other cysts.

Inflammatory lesions

Two dogs, a male 6-year-old Scottish terrier and a male 6-year old Lurcher, had hypophysitis accompanied by moderate bilateral cortical atrophy of the adrenal glands and clinically presenting with hypoadrenocorticism. Both cases had moderate to severe, lymphoplasmacytic inflammatory cell infiltration with loss of pituitary cells (Fig. S7). 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 polyarthritis, vasculitis and anemia. One cat presenting with feline infectious peritonitis (FIP) had perivascular neutrophilic and lymphoplasmacytic infiltrate within the neurohypophysis.

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) had 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 (Fig. 6). 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-diameter, round, mineral laminated concretions within the pituitary capsule, in the neurohypophysis or within acini in the adenohypophysis.

The adenohypophysis from all 23 puppies and young dogs (up to 9-months-old) and a lactating bitch, had a uniform basophilic cell appearance with cytoplasm devoid of granules, and large, vesicular nuclei compared to the adult and elderly dogs (Fig. S8 and S9). This difference was not observed in 5 kittens and young cats (less than 9 months).

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 was unexpected; very similar to the reports in humans with incidentally observed pituitary lesions. The prevalences in our study population might be overestimated for the general dog and cat population, considering that most of the animals were sick animals, with the exception with 8 dogs that were euthanasied due to a severe aggressive behaviour.

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 [36]. In our study, a total of 4 greyhounds and greyhound-cross breeds presented pituitary neoplasia, which 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 frequent 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 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 humans, they are thought to arise from corticotrophs that fail to process the ACTH precursor, pro-opiomelanocortin, into the biologically active 1–39 ACTH [2].

The presence of diffuse, moderate adrenocortical hyperplasia that might suggest an increased production of ACTH was present 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 [24,28].

Only 1 dog had been diagnosed ante mortem with hyperadrenocorticism and had typical lesions of pituitary dependent hyperadrenocorticism, with a very small (1.8-mm-diameter) ACTH-immunoreactive pituitary adenoma that 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 [14].

In our opinion, the significance of the rather arbitrary term micro/macroadenoma based on a cut-off limit of 0.5 or 1 cm to indicate a possible mass effect, used in human medicine [7,19] and adopted in veterinary pathology books [14] and some articles [25,27], is questionable since it does not take into account the variation in size of the pituitary glands in different species and different-sized animal breeds. Radiologists

expect to see lesions ≥2-3 mm in diameter on MRI, depending on the resolution of the device used, and improved technical equipment and increased usage of medical imaging increases the number 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 postmortem examination, and to include the size of the neoplasm when possible.

Every pituitary adenoma had well-defined, strong, uniform, intracytoplasmic ACTH immunereactivity, with almost identical histological morphology: abundant, granular, eosinophilic, cytoplasm that stained uniformly violet with PAS. Human literature reports that corticotroph adenomas have PAS-positive intracytoplasmic granules, attributed to glycolipids, glycoproteins or mucoproteins in the secretory granules or in their membranes [31]. In diagnostic pathologyl settings in which IHC is not available, a strong cytoplasmic granular PAS positivity in a proliferative area with a loss of reticulin fibers can support a diagnosis of ACTH-corticotroph adenoma, bearing in mind that cells producing TSH and FSH/LH also contain PAS-positive granules (but these latter neoplasms have not been reported in dogs) [14]. MSH-secreting adenomas share similar microscopic characteristics with ACTH-secreting adenomas but have strong immunoreactivity for α-MSH, its precursor pro-opiomelanocortin (POMC), and are weakly ACTH-immunoreactive [11,21].

The ACTH non-immunoreactive neoplastic cell population with scant cytoplasm observed within 2 corticotroph adenomas might have been corticotroph cells that have already released their secretory granules. However, a pre-existing, non-neoplastic cell population (suggesting infiltrative growth) or a second neoplastic population of FSH/LH- or TSH-secreting cells is less likely but cannot be ruled out [47].

Unfortunately, we were unable to identify a source of available antibodies for these additional hormones in dogs and cats.

In humans with functional ACTH-secreting pituitary proliferations, non-neoplastic corticotrophs may have 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 [5,23,30], which to our knowledge has not been previously described in small animals. The few nonneoplastic corticotroph cells with strong, PAS-positive, peripheral, intracytoplasmic staining adjacent to 4 canine corticotroph adenomas in our study population, morphologically resemble *Crooke's cells* with peripheral dispersion of PAS positive granules in the cytoplasm and a strong cytokeratin immunolabelling. However, the peripheral hyaline bands were not be easily identifiable on HE although cytoplasmic accumulation of intermediate filaments could be demonstrated on cytokeratin IHC (Fig. 4b).

The Gordon-Sweet reticulin stain was useful to differentiate adenomas from pituitary hyperplasia: hyperplastic lesions retained the acinar pattern of the pre-existing reticulin network whereas in pituitary adenomas the reticulin network is minimal [14,23]. However, there appears to be a continuous spectrum of proliferative lesions between focal (nodular) hyperplasia and adenoma, with a similar prognosis and treatment [36], so distinction between these entities may not be clinically important. The normal pituitary gland of cats is less homogeneous than in dogs, and exhibits great variation in cell populations, which apparently correlates with certain

phases of the reproductive cycle [4]. Thus, the reticulin stain was especially useful to avoid overinterpretation of proliferative lesions in cats.

Pituitary carcinoma was diagnosed in a dog in this study based on the observed necrosis, mitoses, invasion and widespread, destructive, discontinuous growth into the brain [37]. However, we are aware of the discrepancy with other references where metastatic behavior of the tumor is needed to diagnose a pituitary carcinoma [14,24,32,36], and the term "invasive or atypical adenoma" is used instead [14,32]. As a general criterion, destructive infiltration into a neighboring organ is considered a reliable feature that differentiates malignant from benign tumors [16,29], 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 carcinoma by either definition is low in dogs [32,36], with only few cases show metastases [10,32,40]. Only one case of pituitary carcinoma was reported in a cat, with marked bone invasiveness but no metastases [13].

In the 2 cases of pituitary metastases of 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 humans where only 7% of the metastases to the pituitary are symptomatic, with diabetes insipidus, visual field defects, headache/pain and ophthalmoplegia being the most commonly reported symptoms [8]. Distant metastases from extrasellar carcinomas to the pituitary gland such as lymphoma, melanoma, transmissible venereal tumor and adenocarcinomas have been previously reported in dogs [36].

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 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 [42]. 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 [36].

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 [36,42]. 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 the proportion of brachycephalic dogs was higher, this might explain the differences in prevalence.

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 an immune-mediated 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 [1,20,22,33,38,45], usually related to panhypopituitarism and secondary hypoadrenocorticism with adrenocortical atrophy, similar to the cases identified here.

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 [7]. In the affected dog, the mineralization was considered to represent metastatic calcification due to chronic renal failure. To our knowledge, calcification in the pituitary gland has only been described previously together with neoplastic lesions or cysts [15], 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 was interpreted as 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 humans, 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 [6]. In humans, ageing changes such as fibrosis, deposition of amyloid, iron pigment and a decrease in acidophilic and chromophobic cells has been reported [12,39], but none of these changes were not observed in geriatric dogs or cats we examined.

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 in keeping with that described in humans. Most of these lesions were not identifiable macroscopically, so postmortem sampling and histopathological examination of the pituitary gland is needed. 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 humans [9,25]. 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 and which cause undiagnosed functional or clinical.

ACKNOWLEDGEMENTS

We thank the technical diagnostic team of the University of Utrecht, the Pathology team of SVMS of the University of Nottingham and Alan Lasslett for excellent technical assistance.

REFERENCES

1. Adissu H, Hamel-Jolette A, Foster R. Lymphocytic adenohypophysitis and adrenalitis in a dog with adrenal and thyroid atrophy. *Vet Pathol*. 2010;47:1082–1085.
2. Al-Brahim NYY, Asa SL. My approach to pathology of the pituitary gland. *J Clin Pathol*. 2006;59:1245–1253.
3. Cosio C, Sartori E, Garatti M, et al. Prolactinoma in a Dog. *Vet Pathol*. 2017;54:972–976.
4. Dawson AB. The relationships of the epithelial components of the pituitary gland of the rabbit and cat. *Anat Rec*. 1937;69:471–485.
5. DeLellis RA, Lloyds R V, Heitz PU, Eng C. Pituitary tumors. In: *Pathology and genetics of tumours of endocrine organs*. IARCpress; 2004:10–47.
6. Dietrich RB, Lis LE, Greensite FS, Pitt D. Normal MR Appearance of the Pituitary Gland in the First 2 Years of Life. *Am J Neuroradiol*. 1995;16:1413–1419.
7. Ellison D, Love S, Cardao S, et al. Neoplasms in the region of the pituitary fossa. In: Elsevier Mosby, ed. *Neuropathology*. Toronto; 2013:819–834.

8. Fassett DR, Couldwell WT. Metastases to the pituitary gland. *Neurosurg Focus*. 2004;16:8.
9. Freda PU, Beckers AM, Katznelson L, et al. Pituitary Incidentaloma: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* . 2011;96:894–904.
10. Gestier S, Cook RW, Agnew W, Kiupel M. Silent Pituitary Corticotroph Carcinoma in a Young Dog. *J Comp Pathol*. 2012;146:327–331.
11. Goossens MM, Rijnberk A, Mol JA, Wolfswinkel J, Voorhout G. Central diabetes insipidus in a dog with a pro-opiomelanocortin-producing pituitary tumor not causing hyperadrenocorticism. *J Vet Intern Med*. 1995;9:361–365.
12. Hashizume M, Takagi J, Kanehira T, et al. Histologic study of age-related change in the posterior pituitary gland focusing on abnormal deposition of tau protein. *Pathol Int*. 2011;61:13–18.
13. Kimitsuki K, Boonsriroj H, Kojima D, Park C-H. A case report of feline pituitary carcinoma with hypercortisolism. *J Vet Med Sci*. 2014;76:133–138.
14. Kiupel M, Capen C, Miller R, Smedley R. *Histological Classification of Tumors of the Endocrine System of Domestic Animals*. CL Davis DVM Foundation & Armed Forces Institute of Pathology; 2008.
15. Kraft SL, Gavin PR, DeHaan C, Moore M, Wendling LR, Leathers CW. Retrospective Review of 50 Canine Intracranial Tumors Evaluated by Magnetic Resonance Imaging. *J Vet Intern Med*. 1997;11:218–225.
16. Kumar V, Abbas A, Aster J. Neoplasia. In: *Pathology Basis of Disease*. Philadelphia: Elsevier Saunders; 2015:265–340.
17. Lamb CR, Ciasca TC, Mantis P, et al. Computed tomographic signs of acromegaly in 68 diabetic cats with hypersomatotropism. *J Feline Med Surg*. 2014;16:99–108.
18. Longo M, Binanti D, Zagarella PG, et al. A rare case of pituitary chromophobe carcinoma in a dog: clinical, tomographic and histopathological findings. *Open Vet J*. 2016;6:158–161.
19. Maitra A. The endocrine system. In: *Robbins & Cotran Pathologic Basis of Disease*. Elsevier Health Sciences; 2015:1074–1137.
20. McAllister MM. Adenohypophysitis Associated with Sebaceous Gland Atrophy in a Dog. *Vet Pathol*. 1991;28:340–341.
21. Meij BP, van der Vlugt-Meijer RH, van den Ingh T, Flik G, Rijnberk A. Melanotroph Pituitary Adenoma in a Cat with Diabetes Mellitus. *Vet Pathol*. 2005;42:92–97.
22. Meij B, Voorhout G, Rijnberk A. Progress in transsphenoidal hypophysectomy for treatment of pituitary-dependent hyperadrenocorticism in dogs and cats. *Mol Cell Endocrinol*. 2002;197:89–96.
23. Mete O, Asa SL. Clinicopathological Correlations in Pituitary Adenomas. *Brain Pathol*. 2012;22:443–453.
24. Miller MA. Endocrine System. In: *Pathologic Basis of Veterinary Disease*. 2017:682–723.
25. Molitch ME. Diagnosis and Treatment of Pituitary Adenomas. 2017;317:516.
26. Molitch M. Nonfunctioning pituitary tumors. In: *Handbook of clinical neurology*. Elsevier; 2014:167–184.
27. Moore SA, O'Brien DP. Canine pituitary macrotumors. *Compend Contin Educ Vet*. 2008;30:33–40;
28. Myers NC. Adrenal incidentalomas. Diagnostic workup of the incidentally

discovered adrenal mass. *Vet Clin North Am Small Anim Pract*. 1997 Mar;27:381–399.
29. Newkirk KM, Brannick EM, Kusewitt DF. Pathology Basis of Veterinary Disease. In: Zachary J, ed. *Pathology Basis of Veterinary Disease*. Elsevier; 2017:286–322.
30. Oldfield EH, Vance ML, Louis RG, Pledger CL, Jane JA, Lopes MBS. Crooke's changes in cushing's syndrome depends on degree of hypercortisolism and individual susceptibility. *J Clin Endocrinol Metab*. 2015;100:3165–3171.
31. Plant TM, Zeleznik AJ. *Knobil and Neill's Physiology of Reproduction*. Plant TM, Zeleznik AJ, eds. Elsevier Inc.; 2015.
32. Pollard RE, Reilly CM, Uerling MR, Wood FD, Feldman EC. Cross-Sectional Imaging Characteristics of Pituitary Adenomas, Invasive Adenomas and Adenocarcinomas in Dogs: 33 Cases (1988-2006). *J Vet Intern Med*. 2010;24:160–165.
33. Polledo L, Oliveira M, Adamany J, Graham P, Baiker K. Hypophysitis, Panhypopituitarism, and Hypothalamitis in a Scottish Terrier Dog. *J Vet Intern Med*. 2017;
34. van Rijn SJ, Galac S, Tryfonidou MA, et al. The Influence of Pituitary Size on Outcome After Transsphenoidal Hypophysectomy in a Large Cohort of Dogs with Pituitary-Dependent Hypercortisolism. *J Vet Intern Med*. 2016;30:989–995.
35. van Rijn SJ, Grinwis GCM, Penning LC, Meij BP. Expression of Ki-67, PCNA, and p27kip1 in canine pituitary corticotroph adenomas. *Domest Anim Endocrinol*. 2010 May [cited 2017 Sep 26];38:244–252.
36. Rosol T, Grone A, Rosol J, et al. Endocrine Glands. In: *Jubb, Kennedy & Palmer's Pathology of Domestic Animals: Volume 3*. Elsevier; 2016:269–357.
37. Rosol T, Meuten DJ. Tumors of endocrine glands. In: Meuten DJ, ed. *Tumors in Domestic Animals*. Willey Blackwey; 2017:766–833.
38. Rzechorzek NM, Liuti T, Stalin C, Marioni-Henry K. Restored vision in a young dog following corticosteroid treatment of presumptive hypophysitis. *BMC Vet Res*. 2016;13:63.
39. Sano T, Kovacs KT, Scheithauer BW, Young WF. Aging and the human pituitary gland. *Mayo Clin Proc*. 1993;68:971–977.
40. Sarfaty D, Carrillo JM, Peterson ME. Neurologic, endocrinologic, and pathologic findings associated with large pituitary tumors in dogs: eight cases (1976-1984). *J Am Vet Med Assoc*. 1988;193:854–856.
41. Sato A, Teshima T, Ishino H, et al. A magnetic resonance imaging-based classification system for indication of trans-sphenoidal hypophysectomy in canine pituitary-dependent hypercortisolism. *J Small Anim Pract*. 2016;57:240–246.
42. Schiefer B, Hänichen T. On the knowledge and possible significance of hypophyseal cysts in dogs. *Acta Neuropathol*. 1967;7:232–241.
43. Snyder JM, Lipitz L, Skorupski KA, Shofer FS, Van Winkle TJ. Secondary Intracranial Neoplasia in the Dog: 177 Cases (1986-2003). *J Vet Intern Med*. 2008;22:172–177.
44. Troxel MT. Brain tumors. In: *Consultations in feline internal medicine Volume 6*. Saunders/Elsevier; 2010:884.
45. Wolfesberger B, Fuchs-Baumgartinger A, Schwendenwein I, Zeugswetter F, Shibly S. Sudden Death in a Dog with Lymphoplasmacytic Hypophysitis. *J*

Comp Pathol. 2011;145:231–234.

46. Wood FD, Pollard RE, Uerling MR, Feldman EC. Diagnostic imaging findings and endocrine test results in dogs with pituitary-dependent hyperadrenocorticism that did or did not have neurologic abnormalities: 157 cases (1989-2005). *J Am Vet Med Assoc*. 2007;231:1081–1085.

47. Zieliński G, Maksymowicz M, Podgórski J, Olszewski WT. Double, Synchronous Pituitary Adenomas Causing Acromegaly and Cushing's Disease. A Case Report and Review of Literature. *Endocr Pathol*. 2013;24:92–99.

FIGURE LEGENDS

Figure 1. Distribution of pituitary lesions (neoplasia, cysts/cyst-like lesions, inflammation) in dogs of various ages. Only dogs with lesions are included in this figure. Inflamm: inflammation.

Figures 2. Pituitary adenoma, dog. Focal nodule in the pars distalis of neoplastic cells arranged in sheets with fine supportive stroma , Hematoxylin and eosin (HE) (a), with loss of normal reticulin stromal pattern, reticulin stain (b), abundant, violet, uniform, PAS positive cytoplasm, PAS (c) and strong ACTH immunoreactivity, ACTH-IHC (d).

Figure 3. Pituitary carcinoma, dog. An infiltrative and non-encapsulated mass arose from the pars distalis, invading dorsally and discontinuously into the hypothalamus and thalamic region, which histologically show neoplastic cells arranged in tightly packed cords, islets or nests, HE (a), with loss of normal reticulin stromal pattern, reticulin stain (b), which did not show PAS reaction (c), or ACTH immunoreactivity (d).

Figure 4. Pituitary adenoma, dog. a. Clusters of non-neoplastic corticotrophs have intensely PAS-positive granules in the periphery of the cytoplasm with a clear perinuclear area. b. Some of these cells have perinuclear pancytokeratin immunoreactivity.

Figure 5. Pituitary cyst-like lesion, dog. The pituitary gland contains a cystic area without an epithelial lining. HE.

Figure 6. Metastatic mineralization, neurohypophysis, dog. The lesion developed in a dog with chronic renal failure. HE.

Supplementary figure legends

Figure S1. Pituitary carcinoma, dog. Infiltration of neoplastic cells into the thalamus. HE (a), showing moderate amount of fibrous stroma, reticulin stain (b).

Figure S2. Pituitary hyperplasia, cat. Nodular proliferation of neoplastic cells which causes enlargement of the acini, HE (a), with minimal to no visible changes in the normal pituitary stromal network, reticulin stain (b).

Figure S3. Pituitary cyst, dog, containing, pale mucinous fluid lined by ciliated epithelium, HE.

Figure S4: Pituitary cyst, dog, containing, pale mucinous fluid lined by cuboidal, non-ciliated epithelium, HE.

Figure S5: Pituitary cystic area, pars distalis, dog, without an epithelial lining, HE.

Figure S6: Enlarged colloid follicles, cat, pars intermedia, HE.

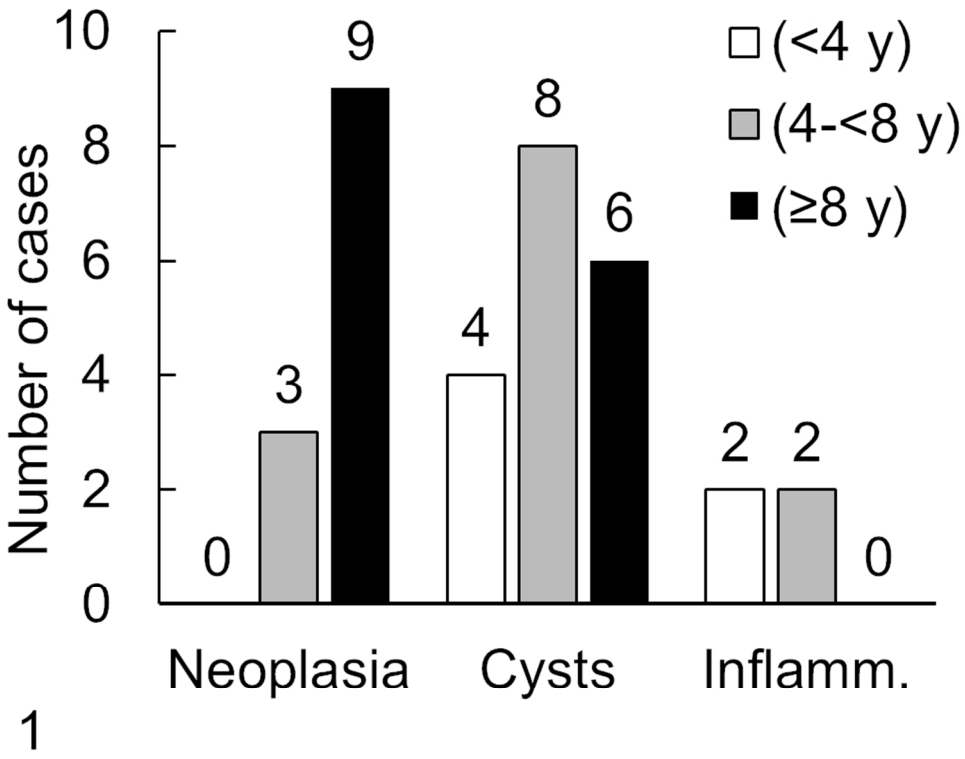
Figure S7: Lymphocytic hypophysitis, dog, HE.

Figure S8: Normal pituitary gland, pars distalis, adult dog, HE.

Figure S9: Normal, pituitary gland, 6 weeks puppy, HE. Note the more basophilic appearance of the pars distalis with a larger proportion of chromophobes and fewer cells containing eosinophilic granules.

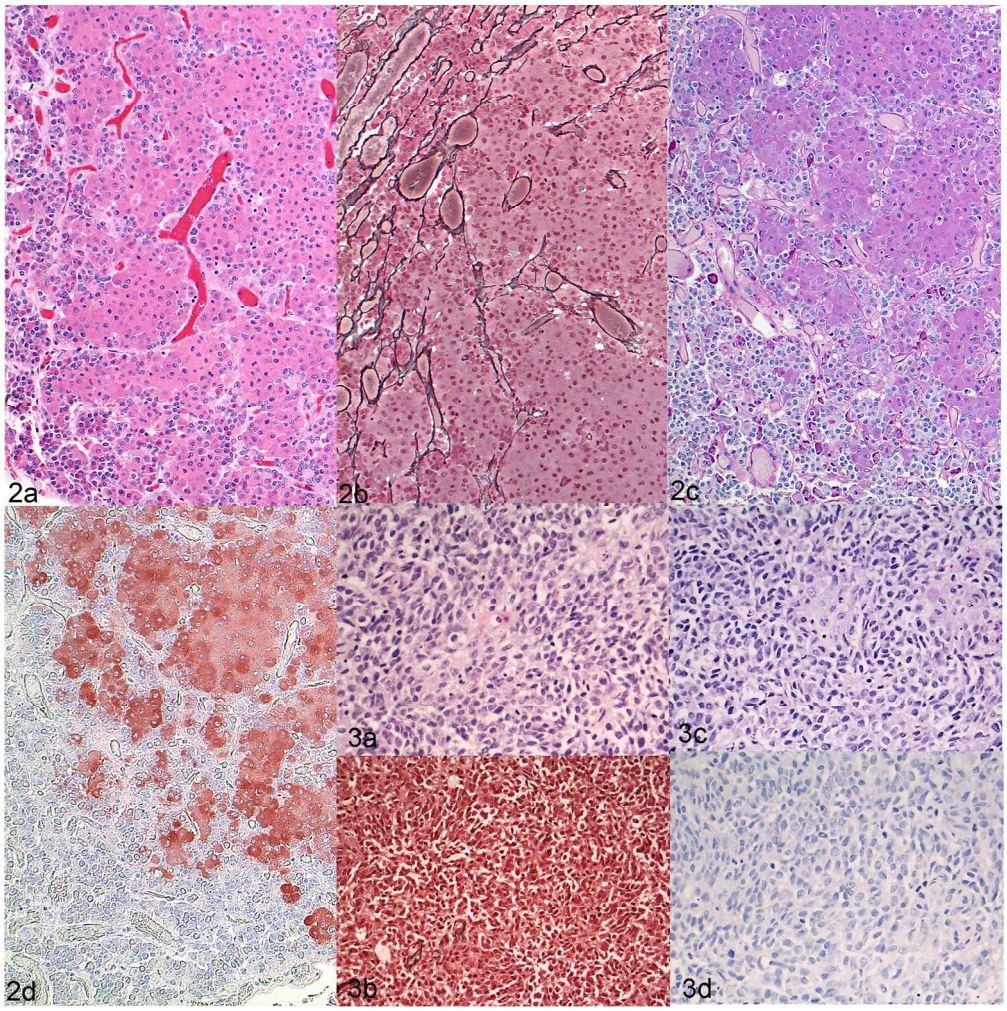
Table 1. Pathological findings in pituitary glands of 136 dogs and 65 cats

Pathological findings	Dogs		Cats	
	Number	%	Number	%
Cystic lesions	18	13.2	8	12.3
Neoplasia/hyperplasia:	12	8.8	1	1.5
Adenoma	11	8.0	0	0
Carcinoma	1	0.7	0	0
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

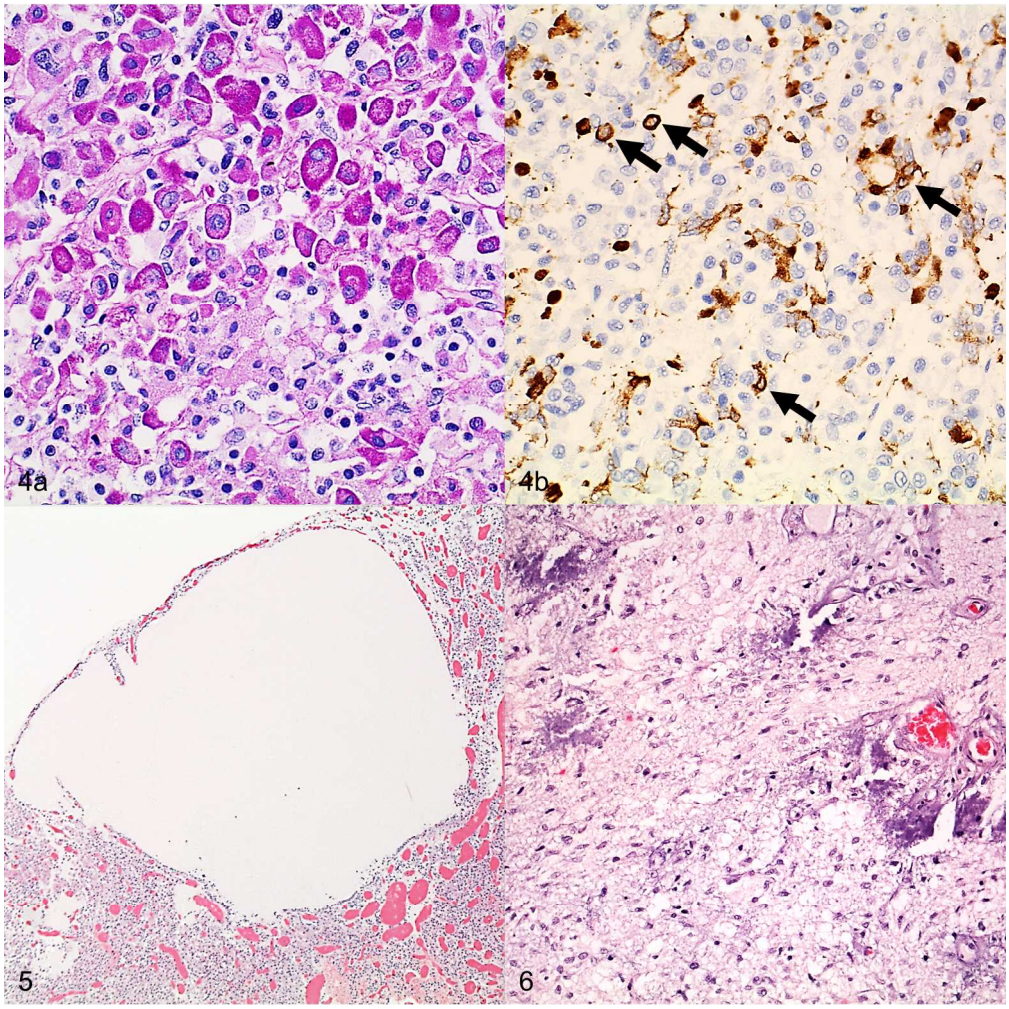


1

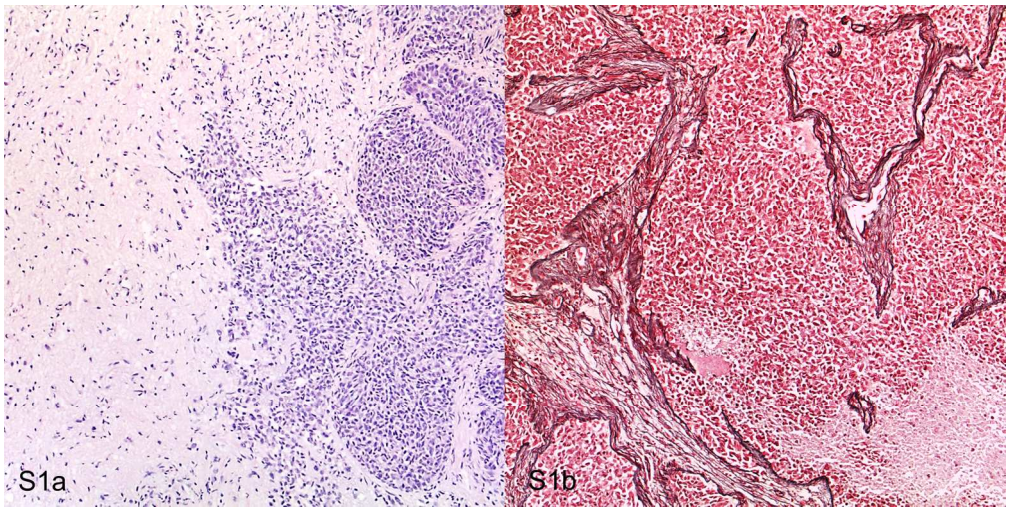
90x72mm (300 x 300 DPI)



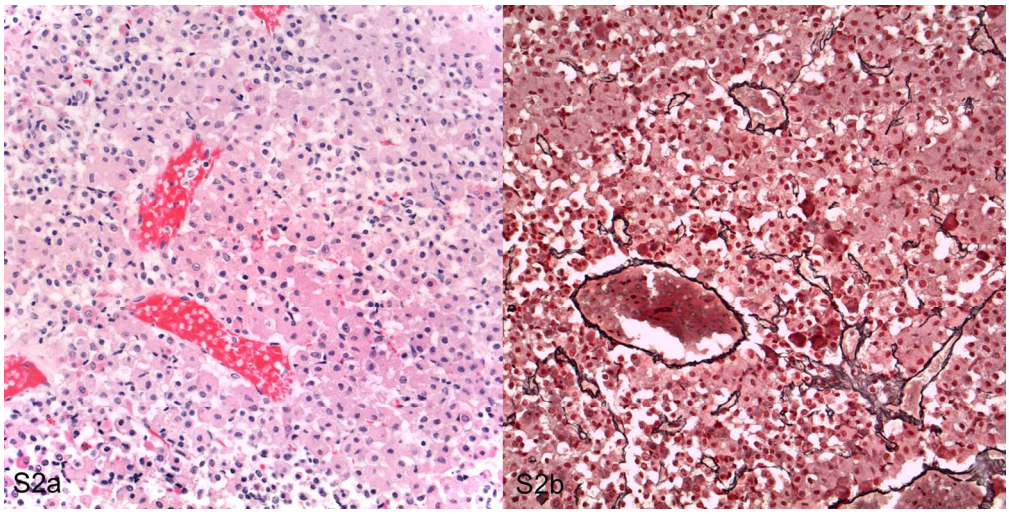
180x180mm (300 x 300 DPI)



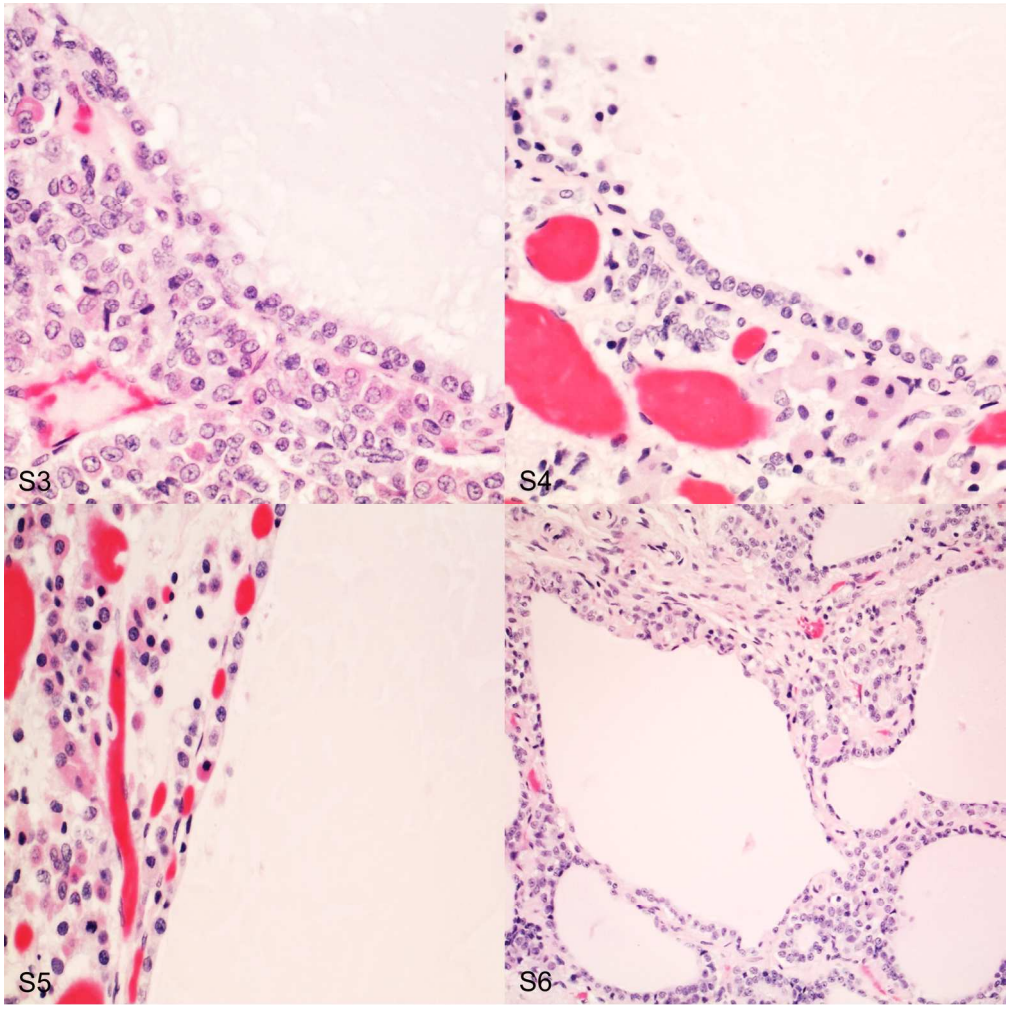
180x180mm (300 x 300 DPI)



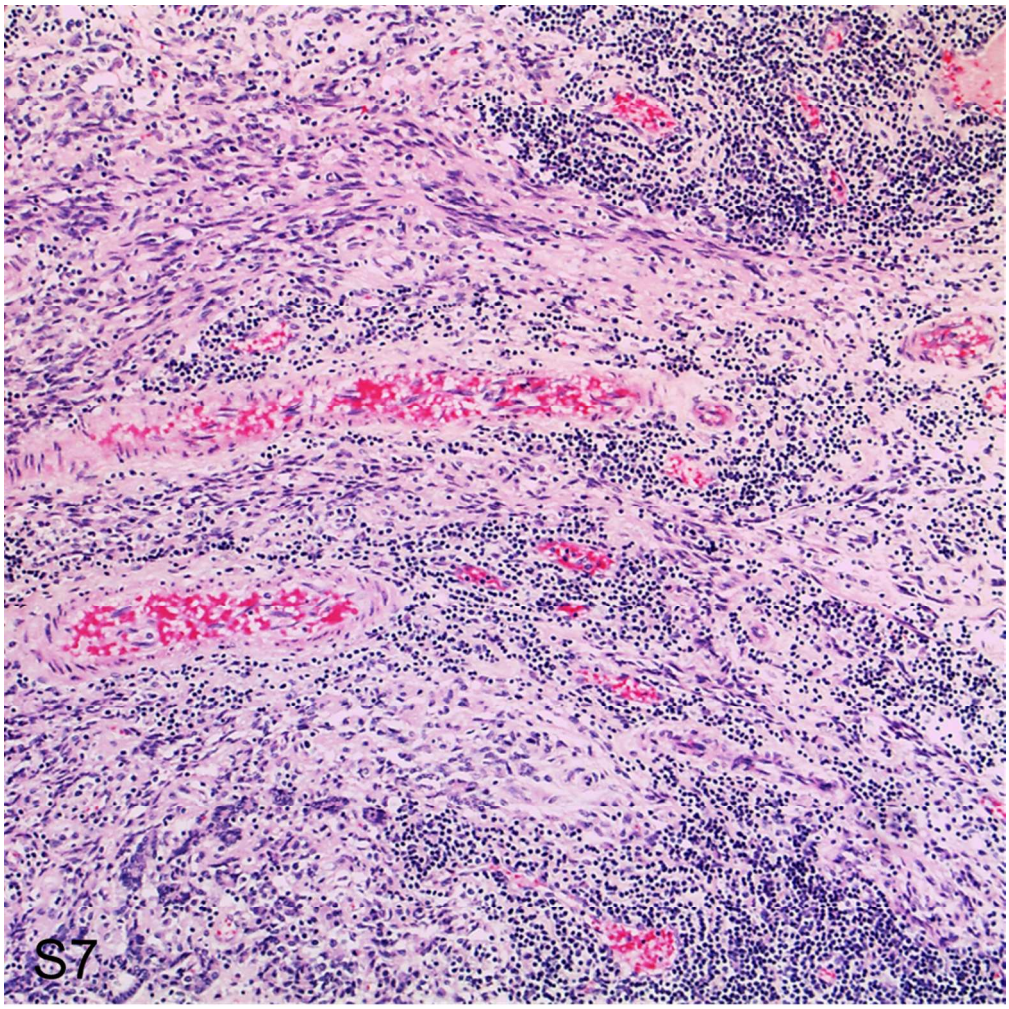
180x90mm (300 x 300 DPI)



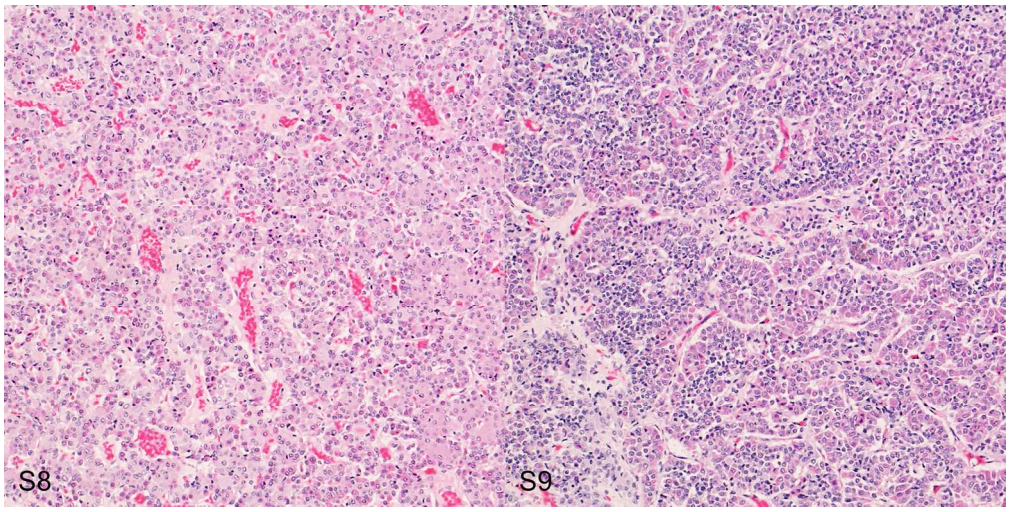
180x90mm (300 x 300 DPI)



180x180mm (300 x 300 DPI)



90x90mm (300 x 300 DPI)



180x90mm (300 x 300 DPI)

Table S1: Supplementary data of cases

ID.	Species	Age	Breed	Gender	Castration	Lesion in pituitary gland	Final diagnosis (Cause of death)
1338	D	6	AMERICAN BULLDOG	M	+	No lesion	Spinal disc herniation
1353	C	12	BIRMAN	F	+	No lesion	Bacterial pneumonia
1356	C	5 M	DOMESTIC SHORT HAIR	M	-	No lesion	Starvation
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 plamocytoma
1402	C	9 M	DOMESTIC SHORT HAIR	M	+	No lesion	Hypertrophic cardiomyopathy
1404	D	5	CHIHUAHUA	F	+	No lesion	Unknown
1405	D	16	STAFFORSHIRE x	M	+	No lesion	Liver rupture-Hypovolemia
1408	D	7 M	BRITISH BULL DOG	F	-	No lesion	Unknown
1411	C	15	DOMESTIC LONG HAIR	F	+	Cysts	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
1433	C	3	DOMESTIC SHORT HAIR	M	+	No lesion	Unknown
1436	D	8 M	GERMAN SHEPERD	F	-	Hypophysitis	Canine distemper
N15-50	C	14	DOMESTIC SHORT HAIR	M	+	No lesion	Pancreatic amyloidosis - D. mellitus
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
N15-196	C	10	EXOTIC SHORTHAIR	M	+	No lesion	Renal failure
N15-198	C	13	PERSIAN	F	+	No lesion	Eye post-surgery complication- aggression
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
N15-216	C	15	DOMESTIC SHORT HAIR	F	+	No lesion	Liver neoplasm rupture - Haemoabdomen
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
N15-252	C	11	BIRMAN	M	+	No lesion	Septicemia
N15-255	D	11 M	LABRADOR RETRIEVER	M	-	No lesion	Hemorrhagic 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	Hemorrhagic enteritis
N15-266	C	12	DOMESTIC SHORT HAIR	M	+	No lesion	Cardiac hypertrophy
N15-268	D	3	BORDER COLLIE	F	-	No lesion	Septicemia
N15-269	D	5	BRIARD	M	+	No lesion	Massive hepatic necrosis
N15-270	C	14	DOMESTIC SHORT HAIR	M	+	No lesion	Hypertrophic cardiomyopathy
N15-273	D	7	YORKSHIRE TERRIER	M	+	No lesion	Intestinal volvulus
N15-274	C	4.5 M	PERSIAN	F	-	No lesion	Herpesvirus interstitial pneumonia
N15-276	D	7	ENGLISH BULLDOG	F	-	Cysts	Brachycephalic syndrome
N15-277	D	3 M	JACK RUSSELL	F	-	No lesion	Cerebellar atrophy
N15-279	C	14	DOMESTIC SHORT HAIR	M	-	Cysts	Rupture pulmonary artery
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 hemorragic enteritis
N15-290	C	12	DOMESTIC SHORT HAIR	M	+	No lesion	Fibroblastic meningioma
N15-291	D	8	LABRADOR RETRIEVER	M	-	No lesion	Neospora cerebellitis
N15-298	D	2	CROSSBREED	M	+	No lesion	Pyothroax - Nocardiosis
N15-301	D	Adult	IRISH WOLFHOUND	F	-	No lesion	Cardiac failure - Severe endocardiosis
N15-302	C	4	BRITISH SHORT HAIR	M	-	No lesion	Hypertrophic cardiomyopathy
N15-303	D	2	GERMAN SHEPERD	F	+	No lesion	Hemoabdomen
N15-304	D	3	AMERICAN BULLDOG x	M	+	Cysts	Starvation
N15-308	C	Adult	RACEDOLL x	F	-	Hypophysitis	Feline infectious peritonitis
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
N15-324	C	8	DOMESTIC SHORT HAIR	F	+	No lesion	Suspected endocrine dysfunction. Diabetes?
N15-327	D	3	GREYHOUND	M	-	No lesion	Asphyxia
N15-340	D	7	FLAT COATED LABRADOR	M	+	No lesion	Hystiocytic sarcoma
N15-342	D	6	SCOTISH TERRIER	M	-	Hypophysitis	Lymphocytic hypophysitis/hypothalamitis
N15-344	C	13	DOMESTIC SHORT HAIR	M	+	No lesion	Feline infectious peritonitis
N15-345	D	6	STAFFORDSHIRE BULL T.	F	-	No lesion	Acute pancreatic necrosis
N15-353	C	1.5	DOMESTIC SHORT HAIR	M	+	No lesion	Diffuse hepatic 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
N15-379	C	20	DOMESTIC SHORT HAIR	F	-	No lesion	Hypertrophic cardiomyopathy
N16-6	D	15	POODLE	M	-	Adenoma	End-stage kidney (renal failure)
N16-8	C	1	DOMESTIC SHORT HAIR	F	+	No lesion	Bacterial enteritis
N16-11	C	3	DOMESTIC SHORT HAIR	F	-	No lesion	Feline infectious peritonitis

N16-13	D	5	ENGLISH SHEEPDOG	M	+	No lesion	Bacterial meningitis
N16-16	D	Adult	SPRINGER SPANIEL	M	-	No lesion	Septicemia
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
N16-26	C	6	BRITISH SHORT HAIR	M	+	No lesion	Feline infectious peritonitis
N16-28	D	9	LABRADOR RETRIEVER	F	+	Cysts	Peripheral nerve sheath tumor (trigeminal nerve root)
N16-29	C	23	DOMESTIC SHORT HAIR	M	+	Hyperplasia	Chronic cardiovascular disorder
N16-30	C	11	DOMESTIC SHORT HAIR	F	+	Cysts	Nasal carcinoma
N16-34	C	16	DOMESTIC SHORT HAIR	F	+	No lesion	Chronic pyelonephritis
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 tumors
N16-47	D	8	GERMAN SHEPERD	M	+	No lesion	Inflammatory bowel disease
N16-48	D	11	JACK RUSSELL	F	-	No lesion	Pyometra
N16-49	D	8 M	AUSTRALIAN SHEPHERD	M	-	No lesion	Septicemia
N16-52	C	5	DOMESTIC SHORT HAIR	F	+	No lesion	No diagnosis
N16-53	C	5 M	DOMESTIC SHORT HAIR	M	-	No lesion	Feline infectious peritonitis
N16-54	C	6	DOMESTIC SHORT HAIR	M	+	No lesion	Restrictive cardiomyopathy
N16-55	D	3	WEIMARANER	M	-	No lesion	Septicemia
N16-56	C	18	DOMESTIC SHORT HAIR	M	+	No lesion	Multifocal calcification
N16-57	D	6	BOXER	F	+	Cysts	Septicemia
N16-58	C	2	DOMESTIC SHORT HAIR	M	+	No lesion	No diagnosis
N16-59	D	5 D	FRENCH BULLDOG	F	-	No lesion	Cleft palate
N16-64	D	3 M	CAVACHON	M	-	No lesion	Parvovirosis
N16-65	C	2	MAINE COON	M	+	No lesion	Ethylene glycol intoxication
N16-70	D	6	SHIH TZU	M	+	No lesion	Congenital porto-systemic shunt
N16-75	D	11	CROSSBREED	M	-	No lesion	Spinal disc herniation
N16-82	D	6	LURCHER	M	+	Hypophysitis	Immunomediated poliarthritis-myocarditis
N16-84	D	3	GREYHOUND	M	-	No lesion	Hemoabdomen - Renal vein rupture
N16-85	D	10	BOXER	M	+	Cysts	Meningioma
N16-86	D	11	COCKER SPANIEL	M	-	Adenoma	Chronic renal failure
N16-87	C	2	DOMESTIC SHORT HAIR	F	+	No lesion	Blunt trauma - Hypovolemic shock
N16-96	D	8	GREYHOUND	M	+	No lesion	Alimentary lymphoma
N16-97	C	5	DOMESTIC SHORT HAIR	F	+	No lesion	Ethylene glycol intoxication
N16-98	C	1	DOMESTIC SHORT HAIR	F	+	No lesion	Ethylene glycol intoxication
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
N16-114	C	16	DOMESTIC LONG HAIR	F	-	No lesion	Cranial trauma
N16-117	C	14	BURMESE	F	+	No lesion	Alimentary lymphoma
N16-118	D	6	DOGUE DE BORDEAUX	M	+	No lesion	Unknown
N16-119	C	18	BIRMAN	F	+	No lesion	Nasal adenocarcinoma
N16-120	C	6	DOMESTIC SHORT HAIR	F	+	No lesion	Feline infectious peritonitis
N16-121	C	9	ORIENTAL SHORT HAIR	M	+	Cysts	Unknown
N16-127	C	2	DOMESTIC SHORT HAIR	M	+	No lesion	Acute pancreatitis
N16-131	D	6	CHIHUAHUA	M	+	No lesion	Granulomatous meningoencephalitis
N16-132	C	8	DOMESTIC SHORT HAIR	M	+	Cysts	Chronic ethylenglycol toxicity
N16-133	C	8	DOMESTIC SHORT HAIR	M	+	No lesion	Ureteral carcinoma
N16-134	C	12	DOMESTIC SHORT HAIR	M	+	No lesion	Bronchiolar carcinoma
N16-137	D	6	GERMAN SHEPERD	F	+	Adenoma	Myocardial degeneration
N16-146	C	5	DOMESTIC SHORT HAIR	M	-	No lesion	Unknown
N16-147	C	11	DOMESTIC SHORT HAIR	M	+	Cysts	Chronic pyelonephritis
N16-148	C	15	DOMESTIC SHORT HAIR	M	+	No lesion	Hypertrophic cardiomyopathy
N16-149	C	12	DOMESTIC SHORT HAIR	M	-	No lesion	Unilateral sciatic neuritis
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
N16-157	C	7	DOMESTIC SHORT HAIR	F	+	No lesion	Lungworm - Eosinophilic pneumonia
N16-163	C	15	BENGAL	F	+	No lesion	Intestinal adenocarcinoma
N16-166	D	7	COCKER SPANIEL	M	-	Cysts	Aortic stenosis
N16-168	C	14	KORAT	F	-	No lesion	Biliary adenocarcinoma
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
N16-188	C	6	DOMESTIC SHORT HAIR	M	+	No lesion	Trauma
N16-191	C	4	SIAMESE	M	-	No lesion	Feline infectious peritonitis
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
N16-222	C	8	DOMESTIC SHORT HAIR	M	-	No lesion	Hepatic necrosis - toxicity
N16-223	D	7	CHIHUAHUA	F	-	No lesion	Astrocytoma
N16-236A	D	2 W	LEONBERGER	F	-	No lesion	Fadding puppy syndrome
N16-236B	D	2 W	LEONBERGER	M	-	No lesion	Fadding puppy syndrome
N16-237	D	9	GOLDEN RETRIEVER	F	-	Cysts	Septicemia
N16-238	D	3	SPRINGER SPANIEL	M	+	No lesion	Intracranial hematoma
N16-239	D	1	BORDER COLLIE	F	+	No lesion	Unknown
N16-253	D	2	LABRADOR RETRIEVER	F	-	Cysts	Intestinal lymphoma
N16-254	C	9	PERSIAN	M	+	No lesion	Unknown
N16-255	D	9	STAFFORDSHIRE BULL T.	M	+	No lesion	Ethylene glycol intoxication
N16-262	C	6 M	DOMESTIC SHORT HAIR	M	-	No lesion	Thyroid carcinoma
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
N16-307	C	11	DOMESTIC SHORT HAIR	F	-	No lesion	Feline pulmonary hystiocytosis
N16-314	C	15	DOMESTIC SHORT HAIR	M	+	No lesion	Ruptured aneurism
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	Astrocytoma
N16-333	D	6	STAFFORDSHIRE BULL T.	F	-	No lesion	Polyoencepathopathy - L-2-hydroxyglutaric aciduria
N16-334	C	9	SIAMESE	F	+	No lesion	Liver necrosis
N16-335	C	14	SIAMESE	M	+	Cysts	Unknown
N16-336	C	1	DOMESTIC SHORT HAIR	F	-	No lesion	Salivary adenocarcinoma
N16-338	D	9	BORDER COLLIE	F	+	Metastasis	Cerebellar metastasis
N16-342	D	9	CROSSBREED	F	+	No lesion	Lung edema
N16-344	D	12 W	SHIH TZU	F	-	No lesion	End-stage kidney
N16-345	C	18	DOMESTIC SHORT HAIR	F	-	No lesion	Feline infectious peritonitis
N16-349	D	14	SPRINGER SPANIEL	M	+	No lesion	Cerebral infarction
N16-361	D	4	ROTTWEILLER	F	-	No lesion	Unknown
N16-364	D	5	STAFFORDSHIRE BULL T.	F	+	No lesion	Glioblastoma
N16-374	C	11	EXOTIC SHORTHAIR	F	+	No lesion	Hypersensitivity reaction type I
N16-375	D	8	LABRADOR RETRIEVER	F	+	Hypophysitis	Adrenal atrophy
N16-384	C	9	DOMESTIC LONG HAIR	F	+	Cysts	Unknown
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
	D=Dog	W= weeks		M= male	-= entire		
	C=Cat	M= months		F= Female	+= Neutered		