Veterinary forensic radiology–Development of a cost-effective and easily performed post mortem computed tomographic angiography protocol

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Declarations

Conflict of interest: No conflicts of interest to disclose

Prior publications: N/A

EQUATOR network checklist: N/A

Abstract

In human forensic medicine, post mortem computed tomography angiography (PMCTA) is routinely utilised in investigations with known superiority for the detection of musculoskeletal and vascular pathology compared to necropsy. In veterinary medicine, there is currently no published PMCTA technique for dogs and cats that is repeatable and can be routinely performed in a referral veterinary hospital. The aim of this study was to develop a veterinary PMCTA protocol that was easy to perform, affordable and requires little additional equipment beyond what is found in a referral veterinary hospital.

This study shows PMCTA can be performed using iohexol mixed with a polyethylene glycol adjuvant and administered via a power pump injector and was successfully demonstrated in 5 dogs and 7 cats.

The cause of death determined from necropsy and PMCTA agreed in 83% of cases and 42%, the cause of death determined on PMCTA was aided by the administration of contrast.

PMCTA outperformed necropsy in the detection of neurological and musculoskeletal pathology, detecting 3.3 times more pathologies. The establishment of an easy-to-perform and affordable PMCTA protocol gives scope for PMCTA use to become widespread in veterinary post mortem investigations, improving the efficiency of post-mortem evaluation.

Highlights: Veterinary post mortem computed tomographic angiography, veterinary post mortem imaging, forensic imaging, post mortem computed tomography

Introduction

In human forensics, post mortem computed tomographic angiography (PMCTA) is regularly used as an adjunctive and alternative to traditional necropsy.[1] In countries such as Japan, Switzerland, and Australia PMCTA has cemented itself as a valid modality in most post mortem investigations.[2-4] In Melbourne at the *Victorian Institute of Forensic Medicine,* PMCTA is the initial step for every cadaver presented to the institution; the PMCTA findings then guide if supplementary procedures are necessary to reach a diagnosis for the cause of death.[2] In humans PMCTA is the method of choice for diagnosing vascular and skeletal pathology when compared to necropsy.[5, 6] In combination with post mortem magnetic

resonance imaging and photogrammetry, PMCTA has the potential to be a component of a completely virtual autopsy.[4]

The veterinary literature on forensic radiology has gradually increased in recent years. Recent studies comparing post mortem computed tomography (PMCT) to necropsy highlight the usefulness of post mortem imaging (PMI) in determining the cause of death.[7, 8] The current published veterinary literature on PMCTA is sparse, consisting of case reports or small case series[9-11] or pilot case series used for establishing techniques in humans.[12, 13] Most of the positive contrast techniques trialled in animals have been conducted by human radiologists for single case reports, and are not practical in a clinical veterinary setting as they require specialised equipment such as a modified heart-lung bypass or expensive contrast agents, both of which are not routinely available.[9, 12, 13] A more cost-effective approach of using air as a negative contrast agent administered by hand pressure has been trialled in goats.[11] Whilst negative contrast agents are cost-effective and technically easy to inject, this study showed air provided no organ enhancement and frequently extravasated from the vessels limiting its benefit as a contrast agent in PMCTA.[11] The use of a highly viscous hydrophilic adjuvant such as polyethylene glycol, in combination with water-soluble iodine-based contrast, is the preferred method for human PMCTA.[14] In the veterinary literature only a single case report exists where this technique is effective in a Brown Howler Monkey.[10]

The use of PMI has many applications in veterinary medicine, from assisting the pathologist to perform targeted necropsies and thereby reducing their workload, to offering an alternative to traditional necropsy that does not involve dissection of the animal.[15]IThere is a

lack of veterinary publications regarding the clinical application of PMI with specific reference to PMCTA.

The first aim of this study was to develop a veterinary PMCTA technique that is economical, easy to perform and requires little additional equipment beyond what is normally found in a referral veterinary hospital. The second was to show that technique is repeatable in cats and dogs. We hypothesize that PMCTA will enhance the detection and characterisation of pathology compared to PMCT and supplement deficits in the necropsy examination.

Materials and Methods

Subjects

The subjects consisted of a convenience sample of 5 dogs and 7 cats presenting to U-Vet Werribee Animal Hospital Anatomical Pathology Service for traditional necropsy between January 2021 and October 2021. Animals were either referred for a necropsy exam after being euthanised within the hospital or presented to the service by their owners after being found to have died at home. Neither group (euthanised in the hospital or died at home) had a definitive antemortem diagnosis. As all the animals were deceased and their owners had consented to a post mortem, a *Notification of Scavenged Animal Tissue* was deemed appropriate in lieu of specific ethics approval by the University of Melbourne ethics committee.

115 The animals included in the study were either frozen and subsequently thawed or fresh,
116 based on the availability of computed tomography (CT) and Anatomic Pathology services.
117 Frozen animals were thawed at approximately 6°C for 24-72 hours depending on the size of
118 the animal and the day of the week.
119 The identity of the animal was confirmed by correlating the signalment and identity patient
120 number on the body bag, and the scanned microchip number with the medical record-
121 keeping system: Rx Works (Covetrus, Australia).
122
123 Technique
124
125 The animals were placed in right lateral recumbency and the left hindlimb was abducted
126 from the body. A surgical incision using a scalpel was made through the cutaneous tissue on
127 the medial aspect of the thigh at the level of the femoral triangle.[16] The deep medial femoral
128 fascia was bluntly dissected and the femoral artery and femoral vein were identified.[16] The
129 right femoral vessels were preferentially catheterised, though if catheterisation was not
130 possible due to pathology or iatrogenic vessel damage, the left was used.
131
132 Once visualised, the fascia surrounding the femoral vessels was pared away extending 2-
133 5cm proximally. For all animals, a catheter was placed within both vessels and secured using
134 a modified Chinese finger trap suture. Depending on the size of the animal 16-24G catheters
135 were used. A range of suture materials was used. Larger suture filament sizes (3-4) tended
136 to lead to difficulty securing the catheter and finer filament (3-0 to 4-0) typically lead to
137 slippage of the catheter during placement, so mid-range suture filament size (0-2) were

preferred. The skin defect was left open and the animal was transported to the CT machine.

The animal was placed in right lateral recumbency and a plain CT was performed using a 16-slice CT scanner (Siemens Emotion, Siemens, Erlangen, Germany). Images were acquired from the nares to the tail. The CT parameters varied based on the size of the animal and the body region of interest. The PMCTA protocol was conducted in accordance with Ross et al technique described for human forensic radiology with a few alterations.[14]

Once the plain post mortem computed tomography (PMCT) was acquired, a giving set was connected to the femoral arterial catheter with a Luer-lock. Non-ionic iodinated contrast iohexol (Omnipaque™350) was mixed with polyethylene glycol 400 (PEG 400-Thermo Fischer Scientific Australia PTY LTD) at a ratio of 1:10.

To achieve an arterial phase contrast study 11ml/kg of the solution was administered through the femoral artery followed immediately by CT acquisition.

The process was repeated for the venous phase, where the giving set was attached to the catheter in the femoral vein and 11ml/kg of the solution was administered, followed by CT acquisition.

In the first two animals, attempts at hand-injection of the iohexol solution were unsuccessful due to the high viscosity of the solution. The iohexol-PEG400 was far too viscous to administer with manual pressure and extruded around the syringe plunger as opposed to passing through the nozzle. Both animals were less than 4kg in body weight. In both instances, the solution was successfully administered with a power injector at a rate of 0.6mls/minute. In subsequent animals, the contrast solution's optimum speed of administration was between 0.6-1.3ml/sec maintaining maximum pressures below 300psi.

Faster injection rates lead to unsustainable pressures within the giving set and lead to rupture of the giving set- catheter junction. Rates of injection were the same for both arterial and venous phases.

In all animals, the contrast was successfully administered with a power pump injector (Salient single injector Imaxeon 2010). The primary author found even at the described lower injection rates the viscosity of the solution could lead to separation at the giving set-catheter junction, which was mitigated by holding the junction with handheld pressure. With slower injection speeds, in larger animals administration of iohexol-PEG400 could take up to 10 minutes per phase of the PMCTA.

All body regions had images reconstructed in bone and soft tissue windows, with lung windows available for the thorax. No attempts to inflate the pulmonary parenchyma were trialled in this study. In all studies, multiplanar reformats (MPR) were generated for the head, neck, thorax, abdomen and pelvis in sagittal, dorsal and transverse planes.

Once the post mortem computed tomography angiography (PMCTA) had been completed the study was interpreted by a radiology resident (A.B.) before the necropsy. A standardised necropsy exam was conducted on all animals under the guidance of a boarded anatomical pathologist. Once the standardised necropsy exam had been completed, the pathologist was given the preliminary PMCTA findings and augmented the necropsy exam to address PMCTA findings as required. The pathologist documented in their report the findings that were added as a result of the PMCTA. Unfortunately, one pathologist failed to differentiate

the findings influenced by the PMCTA in their report. The PMCTA images were then reviewed by a boarded radiologist (M.M or D.T.) blinded to the necropsy findings.

Efficaciousness of PMCTA vascular contrast

Effective contrast administration was determined by measuring an arterial phase increase in contrast enhancement of at least 100 HU within a region of interest (ROI) in the lumen of the aorta at the level of the tracheal bifurcation.

The anatomic extent of contrast perfusion was determined for both the arterial and venous phases separately. Effective arterial phase contrast extended from the femoral artery to the carotid arteries. Effective venous phase contrast extended from the femoral vein to the jugular veins.

If a mass was present the degree of contrast enhancement was quantified for each phase of angiography (venous vs arterial). It was then determined if the addition of contrast aided in a PMCTA diagnosis for the overall cause of death or not, based on the presence or absence of abnormal enhancement of pathology.

Comparing PMCTA and necropsy

To compare imaging to necropsy findings, the number of findings with each method was quantified and classified into body regions. Findings detected with one method and absent on the other were identified and where possible patterns were established.

The cause of death and the four most pertinent findings for each method were grouped into the general disease processes of neoplasia, trauma, inflammatory/infectious, degenerative, toxic/metabolic, congenital, haemorrhagic/ischaemic or no pathology identified. By evaluating the cause of death and the four most pertinent findings between PMCTA and necropsy, the proportion of agreement was compared.

Results

Animals

The study included five dogs and seven cats. The signalment, time to post mortem, cause of death and condition of the body are listed in *Table 1*. Nine animals in the study were euthanised at U-Vet Werribee Animal Hospital and were frozen and then thawed before examination. The period in which the animals were frozen varied from two to eighty-two days. Two animals were found dead, one animal was known to have died within 6 hours before freezing/presentation, and the other was found approximately three-four days post mortem with severe signs of autolysis and putrefaction.

Signalment	Period post mortem (days)	Euthanised or Spontaneous death	Frozen/thawed or Fresh or Putrefied
Canine- Mixed breed- 16yo FN	17	Euthanised	F/T

Feline – Domestic Longhair- 9yo FN	4	Found dead	Putrefied
Feline- Domestic Shortharid- 11yo MN	6	Euthanised	F/T
Canine – Staffordshire terrier- 7yo FE	4	Euthanised	F/T
Feline -Devon Rex- 12yo MN	2	Euthanised	F/T
Canine- Staffordshire terrier- 16yo MN	2	Euthanised	F/T
Feline- Ragdoll-6mo FE	0.5	Euthanised	Fresh
Feline- Domestic Longhair- 11yo MN	2	Euthanised	F/T
Canine- West Highland white terrier-10yo FE	11	Euthanised	F/T
Canine- Staffordshire terrier-2yo ME	0.5	Found dead	Fresh
Feline- Domestic Longhair- 13yo FN	77	Euthanised	F/T
Feline- Domestic Shorthair- 12yo FN	82	Euthanised	F/T

228 *Table 1- signalment of patient and post mortem factors. FN- female neutered, FE-female entire, MN-male neutered,*

229 *ME-male entire*

230

Catheterisation was performed consistently by the same veterinarian (A.B.), and took between 10 to 45 minutes, decreasing with increased experience with an average of under 15 minutes.). The author found it was easier to catheterise dogs than cats, due to cats' smaller vessel size and a large amount of perivascular fat. In cats with prolonged post mortem intervals or signs of autolysis, it was exceptionally difficult to catheterise the femoral artery. In five of these cases, the femoral artery was markedly reduced in size compared to the femoral vein and the vessel wall was friable, resulting in difficulty placing the intra-arterial catheter without tearing the proximal wall of the vessel. In one cat that had been frozen for 72 days prior to PMCTA, after tearing of the femoral artery wall at the level of the femoral triangle, the vessel was attempted to be catheterised proximally, though instead a subsidiary of the femoral vein was mistakenly catheterised, resulting in two venous-and no arterial phases.

Effective contrast administration

In 7/12 animals the extent of contrast administration was successful reaching both the jugular veins and carotid arteries in all dogs and 2/7 cats. In three cats the contrast only reached the jugular veins and in two cats the contrast did not move outside of the abdominal vasculature (*Table 2*). The extent of contrast passage is shown by the opacification of the facial and cerebral arteries in *Figure 1.*

In all animals in which the contrast reached the carotid arteries (7/12), the degree of enhancement was deemed effective.

Table 2		
	Aortic lumen increase >100HU	Aortic lumen increase <100HU
Contrast reached the jugular veins and carotid arteries	7	0
Contrast reached the jugular veins only	0	3
Contrast in neither the jugular veins or carotid arteries	0	2
	7/12	5/12

Table 2: The effectiveness and extent of contrast passage.

Correlating and comparing PMCTA and necropsy

The PMCTA-determined most likely cause of death and necropsy final diagnosis for each case are shown in *Table 3*. The cause of death established on PMCTA and necropsy agreed for the disease process in 83% of cases (10/12). The two most pertinent findings for each PMCTA examination were identified and the primary differential diagnosis for disease process was compared with the post mortem diagnosis. There was an agreement between PMCTA and histologic diagnosis of disease process for the two most pertinent CT findings in

264 75% of findings (18/24). For the third and fourth most pertinent findings there was reduced
265 agreement between PMCTA and necropsy with an agreement in 42% of findings (10/24).
266
267

Table 3			
Signalment	Primary differential for cause of death on PMCTA	Cause of death on necropsy	Agreement between PMCTA and Necropsy
Canine 16yo FN	Neoplasia- Splenic neoplasia- likely haemangiosarcoma.	Splenic Haemangiosarcoma	Agree
Feline 9yo FN	Trauma - Traumatic sacrocaudal luxation and sacroiliac subluxation	Traumatic sacrocaudal luxation and sacroiliac subluxation	Agree
Feline 11yo MN	Neoplasia- Pancreatic neoplasia (adenocarcinoma or lymphoma) and metastases	Pancreatic adenocarcinoma with mesenteric metastases	Agree

Canine 7yo FE	Toxic/Metabolic- Hypoattenuating hepatomegaly most consistent with hepatic lipidosis	Toxic hepatopathy with hepatic lipidosis	Agree
Feline 12yo MN	Neoplasia- Bilateral renal and intestinal neoplasia (likely lymphoma)	Renal and jejunal lymphoma	Agree
Canine 16yo MN	Degenerative/Traumatic- Intervertebral disc disease	Coxofemoral osteoarthritis	Disagree
Feline 6mo FE	Inflammatory/infectious- Pleural effusion likely pyothorax	Pyothorax	Agree
Feline 11yo MN	Traumatic – Head trauma	Head trauma	Agree
Canine 10yo FE	Neoplasia- Hepatic mass and abdominal lymphadenopathy	Hepatic haemangiosarcoma	Agree
Canine 2yo ME	Infectious/Inflammatory- Pneumonia	Pneumonia and Sepsis	Agree
Feline 13yo FN	Neoplasia- Nasal cavity and facial mass likely squamous cell carcinoma	Nasal cavity and facial squamous cell carcinoma	Agree

Feline 12yo FN	Neoplasia- Small intestine and caecal thickening likely lymphoma.	Inflammatory enteritis and typhlitis	Disagree

Table 3: Determining agreement between the most likely general disease processes *on PMCTA and necropsy final diagnosis.*

The primary pathology had contrast enhancement or pathological absence of enhancement in 42% of animals (5/12). For the four most pertinent findings, contrast aided the identification of 32% of findings (15/48).

Due to the large variation of PMCTA and necropsy findings in a small population, the findings were grouped by general body regions. *Table 4* provides a list of the findings grouped into musculoskeletal and neurological, abdominal parenchymal and thoracic parenchymal.

Table 4		
Findings	PMCTA	Necropsy
Neurological and Musculoskeletal	46	14
Thoracic parenchymal	11	12

Abdominal parenchymal	32	28

Table 4: Findings grouped by body system, comparison between necropsy and PMCTA.

PMCTA was superior to necropsy in the identification of all musculoskeletal and neurological findings, identifying 3.3 times more pathological findings, including incidental and crucial findings. The number of findings identified on both thoracic and abdominal parenchymal structures was similar between the methods. Moderate to severe lymphadenopathy was more consistently identified on PMCTA compared to necropsy.

The two major pathologies which were missed with PMCTA, but identified with necropsy were associated with vascular mural nodules. In one case an incidental chemodectoma in the wall of a pulmonary artery was not seen in a dog with concurrent diabetic ketoacidosis and mammary neoplasia. In the second case where a dog was euthanised for a haemabdomen, PMCTA identified a liver mass, abdominal lymphadenopathy and pulmonary metastases, but failed to identify a right auricular nodule found on necropsy. The second dog was found to have a primary hepatic haemangiosarcoma with concurrent right auricular haemangiosarcoma. It was not possible to visualise the nodule in both animals on retrospective reviews of the PMCTA.

A common PMCTA finding in post mortem intervals longer than 48 hours was irregular margins and mildly heterogeneous contrast enhancement of the kidneys. In cases where the kidneys had normal size and normal general parenchymal structure, the changes were attributed to autolysis. As a result of attributing the altered appearance of the kidneys to

post mortem change, PMCTA failed to identify a case of infectious nephritis and a case of glomerulonephritis.

Due to variations in adherence to the research protocol by the pathologists, it was not possible to document how many pathological findings were attributed to the addition of PMCTA. As a result comparison between the proportion of standardised necropsy and PMCTA-augmented necropsy findings was not possible in this study.

Comments on findings of PMCT examinations

Commonly on PMCTA examinations there was marked diffuse heterogeneous contrast enhancement of the musculature, most prominent in the epaxial muscles. The muscular contrast enhancement was more conspicuous with shorter post mortem intervals. This was investigated with histological samples in the most severe case, where no abnormalities were noted.

In four animals(two cats and two dogs) soft tissue attenuating material (30-100HU) with a meniscus was seen in the dependent portion of the tympanic cavity on PMCTA. No other concurrent imaging findings of middle ear pathology were seen in any case and no necropsy examination reported findings associated with the middle ears.

Other common incidental findings identified on PMCTA that did not correlate to necropsy pathology include luminal soft tissue attenuating material in nasal and oral cavities and the trachea and regurgitant gastric contents in the oesophagus and oral cavity in animals with no antemortem signs of regurgitation.

Discussion

This is currently the largest and only case series on PMCTA conducted in veterinary patients and the only study to compare veterinary PMCTA with necropsy. This study validates a veterinary PMCTA protocol that is easy to perform, repeatable and cost-effective. PMCTA was shown to have a high level of agreement with necropsy for the cause of death (83%) and first and second-listed pathologies on a necropsy report (75%). PMCTA out-performed necropsy in the identification of musculoskeletal and neurological pathology such as intervertebral disc disease and osteoarthritis. PMCTA did not identify two instances of vascular mural lesions, a chemodectoma and a right auricular haemangiosarcoma. The addition of contrast to the PMCT aided the identification of 42% of the causes of death and 32% of all pathology identified. At the Victorian Institute of Forensic Medicine (VIFM), PMCTA is the initial step for all cadavers presenting at the institute, based on the PMCTA findings it is then determined if there is a need for targeted or full necropsy or toxicological examination or not. The use of this veterinary PMCTA protocol has the potential to become the first step of veterinary post mortem examinations.

Technique

This PMCTA protocol was easy to perform with the time from catheterisation to completion of the examination performed within 30-60 minutes. In most cases the catheterisation process was simple. Limitations of the catheterisation technique were mainly due to autolysis and prolonged post mortem interval and the technical learning of a new technique. There was increased difficulty in catheterisation in cats with prolonged post

mortem intervals or with autolytic changes. This was likely more notable in cats due to the smaller sizes of the feline vessels compared to canines. The author believes the limitations would be reduced with increased expertise in this procedure. No additional equipment was acquired beyond what was normally found at our institution to administer the contrast solution. The suture, vascular catheter and giving set were the same as what was used routinely in practice; to reduce costs expired suture material and previously used giving sets were used. The inability of hand pressure to inject the contrast solution was not unexpected. Correspondence with authors of a case report with a similar technique indicated similar issues.[10] The viscosity of the solution, therefore, limits this technique to situations where a power pump injector is available. At our institution, the power pump injector was the same machine that is used for all computed tomography angiography examinations.

Post mortem angiographic protocols using polyethylene glycol and an aqueous contrast agent are well-established in human medicine.[18] The high viscosity of polyethylene glycol and hydrophilic nature means it conjugates well with the iohexol and prevents extravasation and over-enhancement of the soft tissue structures.[18] Polyethylene glycol has low-level toxicity and high chemical stability,[19] we stored the solution at room temperature and handled it with minimal precautions (wearing gloves and a face mask) as per the chemical safe handling guidelines; without complications. The concentrations of the iohexol: PEG mixtures used for PMCTA examinations have been reported between 1:10-1:20 in the human and veterinary literature.[10, 20] In our study due to the large volumes of contrast mixture and slow injection rate we chose to use the mixture with a lesser volume. Adequate vessel filling with contrast and parenchymal contrast enhancement was seen with this ratio

of the mixture in 7/12 of the animals. In the remaining five animals the difficulties in catheterisation and autolytic changes[21] were most likely responsible for the lack of contrast passage as opposed to the mixture itself. Contrast enhancement in a venous and/or arterial phase was repeatably achieved in 10/12 animals. While this is a lower proportion of animals than we initially anticipated, we believe the proportion of effective contrast administration would increase as experience and familiarity with the technique are gained.

While it was initially difficult in our country to acquire a smaller size volume (50L) of PEG 400 to perform the study, once acquired the solution was relatively easy to handle and affordable: with an approximate cost of $160 AUD for the 12 PMCTA's or on average an additional cost of $13AUD/examination. By increasing the frequency of performing these examinations and buying larger volumes costs could be further reduced.

The authors would like to stipulate that the workflow of the technique between the radiology and pathology departments was solely designed for a research purpose and is not applicable to clinical practice. In clinical practice, the radiologist and pathologist must work cohesively. Ideally both the radiologist and pathologist would be present at the time of femoral catheterisation, so the pathologist can be aware of the iatrogenic alterations to the animal in catheterisation- this would be pertinent in legal cases to prevent cross-contamination of the animal by the radiologist. The animal would then undergo PMCTA and return to the pathology department with a preliminary or finalised PMCTA report available to the pathologist. The pathologist would conduct the post mortem ensuring to address all of the PMCTA findings. For PMCTA to be most effectively utilised in the veterinary profession a harmonious and united working relationship between the radiology and pathology professions is needed.

Pathology on PMCTA

The importance of contrast to a PMCT examination is shown by 42% of the cause of death diagnoses influenced by contrast enhancement. The addition of contrast to a PMCT examination increased the breadth of cases in that post mortem imaging can be applied. In a previous retrospective study from the same institution, post mortem imaging (radiographs and PMCT) was found to reach a diagnosis of the cause of death in 57% of cases and of those 85% had a traumatic cause.[8] This is in contrast to the current PMCTA study where pathology accounting for the cause of death was identified in all cases and there was an agreement with necropsy in 83% of cases. Only two of the animals in this study had a traumatic cause of death. The large discrepancy in the percentage of traumatic causes of death between the previous retrospective study and the current study is most likely due to PMCTA having increased diagnostic prowess in non-traumatic causes of death. The variation between the two studies' populations may also be partly responsible for this discrepancy, as in the previous study 86% of cases were requested for medicolegal reasons, as opposed to the current study which represents a cross-section of the general population of animals presenting for necropsy.[8]

The addition of contrast to the CT examinations not only aided in the identification of pathology that is contrast-enhanced but also in the identification of lesions due to the pathological absence of contrast enhancement. In one case the addition of contrast enabled the visualisation of strong arterial contrast-enhancing parietal pleural plaques in a young cat enabling an infectious cause such as pyothorax to be placed as the primary differential for

the effusion and cause of death. Pathological focal lack of contrast enhancement was able to identify a nodule in the liver found on necropsy to be a haemangiosarcoma in a dog with a haemabdomen. The nodule did not bulge from the liver capsular margins and was isoattenuating with the surrounding parenchyma on the pre-contrast images.

Ventilated pulmonary PMCTA are advocated in human medicine to overcome atelectasis and livor mortis common sequelae post mortem.[22] Recent studies in veterinary patients have established references for the ideal ventilation pressure and documented its value in animals within 180 minutes post mortem.[23] Conducting ventilated pulmonary PMCTA was not one of the aims of this study, but as a result of its absence, pulmonary pathology may have been underreported on PMCTA. In our case series, this statement was not supported by necropsy, which identified no further pulmonary pathology than that seen on PMCTA. The addition of contrast to the PMCT did enable differentiation of atelectatic lung with and without pathology. Non-pathological atelectatic lung appeared on PMCTA as a strongly homogeneous contrast-enhancing tissue with the exception of the bronchial tree. Comparatively, when pathology was evident there was a loss of the homogeneity of contrast enhancement and there was either a diffuse heterogeneous enhancement or focal areas with increased or decreased enhancement. This is similar to what has been described in human radiology for discriminating atelectatic lung from pneumonia.[24]

Comparison between necropsy and PMCTA

In agreement with a previous study,[17] no artifacts or complications from the addition of polyethylene glycol and iohexol were noted on necropsy and histological examination.

In the two cases in which there was disagreement between necropsy and PMCTA, PMCTA did identify the pathology that was responsible for the cause of death. A case where the cause of death differed between necropsy and PMCTA was a 12-yo cat who was euthanised for weight loss and noted on ante mortem ultrasound to have diffuse intestinal wall thickening. PMCTA identified, in this case, diffuse small intestinal wall thickening and marked caecal wall thickening with strong arterial contrast enhancement (*Figure 2)* and intra-abdominal lymphadenopathy. Based on the PMCTA findings a diffuse neoplastic cause was considered the most likely differential diagnosis, such as lymphoma or mastocytosis; while necropsy agreed with all of the PMCTA findings histopathology found the definitive diagnosis to be inflammatory enteritis and typhlitis. Secondary differential diagnoses of PMCTA were listed as inflammatory bowel disease (IBD) and feline eosinophilic sclerosing fibroplasia. The design of this study where the cause of death was categorised by disease processes, meant that while both methods agreed that intestinal thickening and lymphadenopathy were the cause of antemortem signs that lead to euthanasia, the two methods still disagreed based on PMCTA's ranking of differentials. It is not surprising that there is difficulty differentiating diffuse intestinal lymphoma from IBD on PMCTA, given that ante mortem certain imaging features can be suggestive for one or the other though ultimately histology is needed.[25]

The ability of PMCTA to identify substantially more neurological and musculoskeletal pathology than necropsy is consistent with what is reported in the human literature. [5, 6] The preference of PMCTA to necropsy for diagnosing such pathology is illustrated by the final case where there was a discrepancy between the cause of death diagnosed on the two methods. An eight-year-old dog was euthanised for progressive hindlimb ataxia. Necropsy

attributed the cause of death to coxo-femoral osteoarthritis. PMCTA identified extruded intervertebral disc material at the T12-T13 intervertebral disc space and intervertebral disc protrusion at L7-S1 (*Figure 3)*. Ataxia is associated with loss of conscious proprioception secondary to spinal cord compression/injury or with dysfunction of the cerebellum or vestibular system.[26] Given the necropsy findings do not explain the ante mortem clinical signs and an extruded intervertebral disc in the thoracolumbar spine would,[26] the author surmises that the necropsy missed the cause of death in this case. In this study when the same pathology was identified in both methods there was a trend for a necropsy to underestimate the extent of musculoskeletal and neurological findings. An example of this was in a cat with facial trauma, necropsy identified bruising and haemorrhage within the temporalis muscle and missed a depressed skull fracture associated with intracranial haemorrhage and contrast-enhancing meninges (*Figure 4*). This is in agreement with the human literature where the greatest value of the addition of PMCT to a post mortem examination is in the identification of musculoskeletal injury.[6]

The detection of enlarged lymph nodes on PMCTA was superior to necropsy. Necropsy frequently missed enlarged lymph nodes or identified one enlarged lymph node, but failed to identify enlargement of secondary draining lymph nodes. The increased sensitivity of veterinary PMCTA in the identification of enlarged lymph nodes is paralleled in the human literature.[27] The explanations in the human literature for the discrepancy are attributed to the higher vigilance of radiologists for enlarged lymph nodes.[27] In veterinary radiology, especially in feline radiology (where cats have a more uniform body size) there are established CT references for each lymph node size.[28-31] This allows for increased identification of subtly or mildly enlarged lymph nodes that could be overlooked on

necropsy. In most cases, the pathologists were not made aware of all enlarged lymph nodes on the preliminary PMCTA findings. To enable confirmation that these lymph nodes are histologically abnormal, full PMCTA interpretation would be needed to guide necropsy. Due to time and workflow constraints, a complete PMCTA report prior to the necropsy was not always possible.

Human forensics report PMCTA is the method of choice for diagnosing vascular pathology.[5] It was therefore surprising that in our study PMCTA failed to identify two vascular mural nodules, even on retrospective review. In humans, it is reported ante and post mortem thrombi can be differentiated from each other, with ante mortem thrombi adhered to the vascular walls compared to post mortem thrombi which will separate and be surrounded by contrast.[5] In our study a large number of thrombi was seen within the lumen of the vasculature, none of which could be separated from the vascular walls by contrast. A large amount of adhered intraluminal thrombi prevented recognition of both the vascular mural nodules. It is implausible that this number of thrombi were present ante mortem in all cases and this was supported by the necropsy findings. One possible theory is that feline and canine thrombi do not separate from the vascular walls as easily post mortem, however this is not substantiated. Another potential explanation is that a larger volume of contrast or contrast injected at faster rates in humans could have enabled the separation of the thrombi from the vascular walls. There are no reports in the human or veterinary literature of differing doses of contrast affecting the appearance of post mortem thrombi.

Common PMCTA findings of unknown significance

The published normal radiographic appearance of post mortem canine thorax and abdomen and feline abdomen have been shown to have a wide range of variation in temperature- and time-controlled environments.[32-34] Normal PMCT and PMCTA findings are not published in the veterinary literature. We know that following death the body undergoes rigor mortis, autolysis and putrefaction.[35] The onset of each of these stages is influenced by temperature, season, altitude, body fat content, cause of death, hair length and clothing.[35] Without normal references, veterinary PMCTA interpretation is prone to error. Some findings such as regurgitant material in the oesophagus, oropharynx, laryngopharynx and trachea can be presumed normal post mortem change in the absence of signs of aspiration pneumonia or regurgitation in the clinical history. The significance of other findings is not as clear. We have discussed below common findings we found across multiple animals that did not correlate with necropsy findings and in this study were presumed normal post mortem changes.

Prominent heterogeneous contrast enhancement of the epaxial musculature was noted in many animals in our study, most notable in dogs with shorter post mortem intervals. Histopathology collected from the animal with the most severe changes revealed no evidence of pathology. While underlying pathology such as myositis, rhabdomyolysis or neoplasia cannot be excluded without histology in each case, given the finding was present in most animals it seems unlikely it is pathological. Interestingly in human forensic radiology, the use of polyethylene glycol as a carrier agent is associated with over-enhancement of the musculature of the neck.[18] The aetiology of the excessive enhancement of the neck musculature in humans is not published,[18] but a common mechanism could be responsible

for the heterogeneous contrast enhancement seen in the epaxial musculature in the animals in our study.

In four of the animals in our study dependent soft tissue attenuating material with a meniscus in the tympanic bullae was seen on PMCTA. In none of these patients were there associated changes in osseous bullae or associated soft tissues on PMCTA and no pathology was identified on necropsy. This finding is consistent with a previous study on 20 cats which reported tympanic bullae effusions were commonly seen on PMCT but did not correlate with necropsy findings.[7] A veterinary cytology and histology article in dogs has reported on post mortem examination of five dogs with and without tympanic bullae effusions there was evidence of submucosal congestion and in 2/3 of the cases with effusion, there was dilation of the submucosal glands.[36] It is not known whether the middle ear effusions in these four cases represent a normal post mortem change, subclinical otitis media or primary secretory otitis media. Further studies including histological examination of the tympanic bullae lining are needed to ascertain the significance of the effusion as pathology or post mortem change.

Limitations

This study is limited by the small sample size. The use of both dogs and cats of varied ages, sizes and breeds, while representing an accurate cross-section of the workload of the anatomical pathology team, it is a heterogeneous population. The heterogeneity meant that catheter size, the volume of contrast media and injection pressure could not be

564 standardised across the population. Variation and lack of control of the post mortem
565 interval and environment further increased variation in normal post mortem changes.
566
567 In addition to the small sample size, only 7/12 of the animals in this study had an adequate
568 extent of contrast administration. Through this failure, the authors were able to identify
569 common technical errors and signalments where contrast administration was challenging.
570 This information can be applied in future situations where the time and cost of contrast, and
571 risk of a non-diagnostic study may negate PMCT being pursued, for example in a cat with
572 marked autolysis and putrefaction.
573
574 **Conclusion**
575
576 This study validates a veterinary PMCTA protocol that is easy to perform, cost-effective and
577 requires little equipment beyond what is found in a referral veterinary hospital. This
578 technique was repeatable to achieve some contrast enhancement with minimal artefacts in
579 12 dogs and cats. The technique was able to be performed with an adequate breadth of
580 contrast in 7/12 animals with varying degrees of post mortem periods. This is currently the
581 largest and only case series of veterinary PMCTA in the literature. The addition of contrast
582 to the PMCT enhanced the detection of pathology and influenced the cause of death
583 diagnosis in 42% of cases. The use of PMCTA in a veterinary clinical setting is incipient but
584 exciting. PMCTA combined with a unified approach between radiologists and pathologists
585 has the scope to increase the sensitivity and specificity of veterinary post mortem
586 examinations. Around the globe in human forensic medicine, PMCTAs are common practice
587 in post mortem examinations and are known to be superior to necropsy in the detection of

musculoskeletal pathologies, a finding echoed in our study. This study provides guidelines for a technique by which future PMCTAs can be performed and foundation knowledge of pathological and normal PMCTA findings to be explored further. This technique has the scope to become routine practice for PMCTA examinations and to increase the prevalence of PMCTA which is used in veterinary post mortem investigations.

List of Author contribution:

Category1

a) Conception and design: Adrian Bryce, Marjorie Milne and Julien Dandrieux
b) Acquisition of data: Adrian Bryce
c) Analysis and Interpretation of data: Adrian Bryce, Zoe Lenard and Yuchi Chen

Category 2

a) Drafting the article: Adrian Bryce
b) Revising the article for Intellectual Property: Adrian Bryce, Marjorie Milne, Julien Dandrieux, Yuchi Chen and Zoe Lenard

Category 3

a) Final approval of the Completed Article: Adrian Bryce, Zoe Lenard, Julien Dandrieux, Yuchi Chen, Marjorie Milne

Acknowledgement to Dr Dayle Tyrrell for her assistance in the design and acquisition of data and Mr Kane Wilson for his assistance in the image acquisition.

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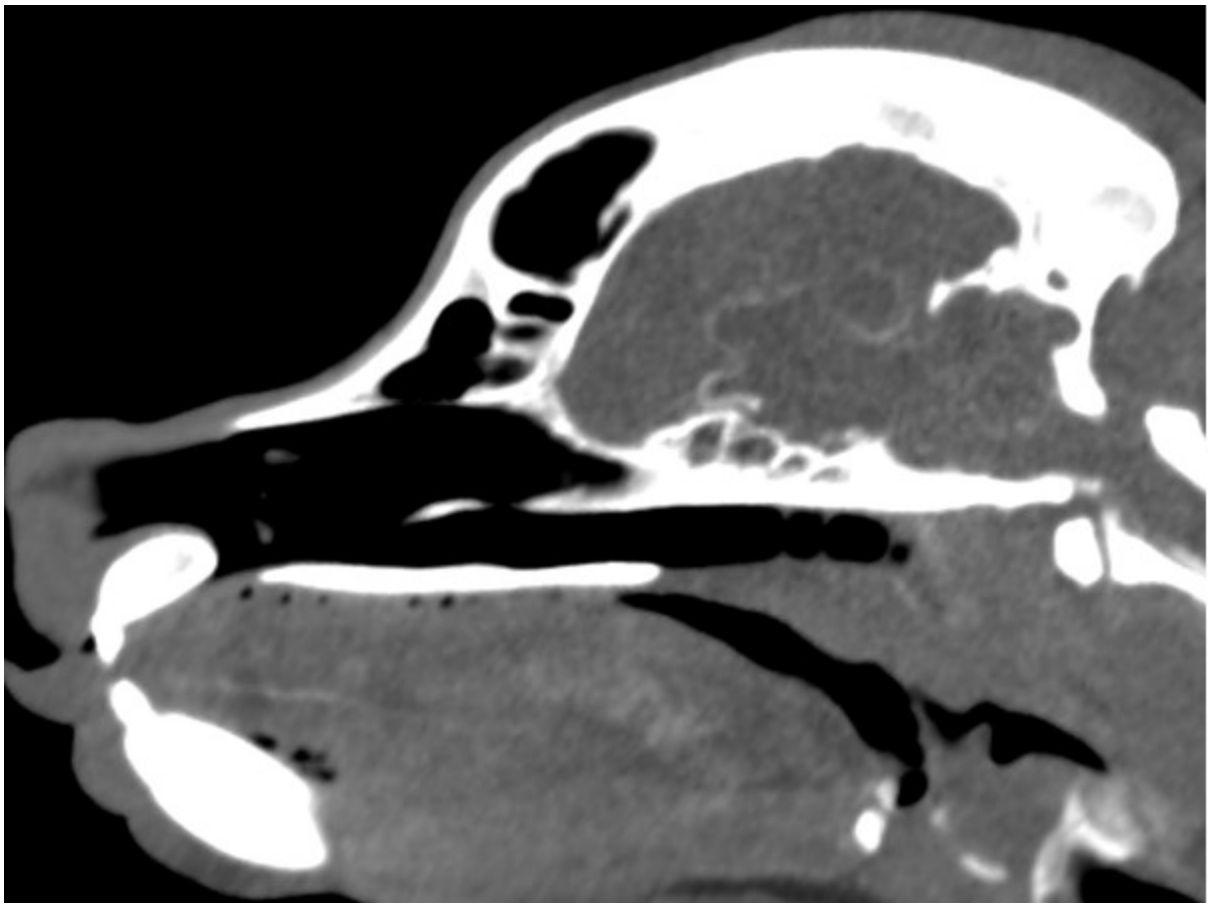
a)



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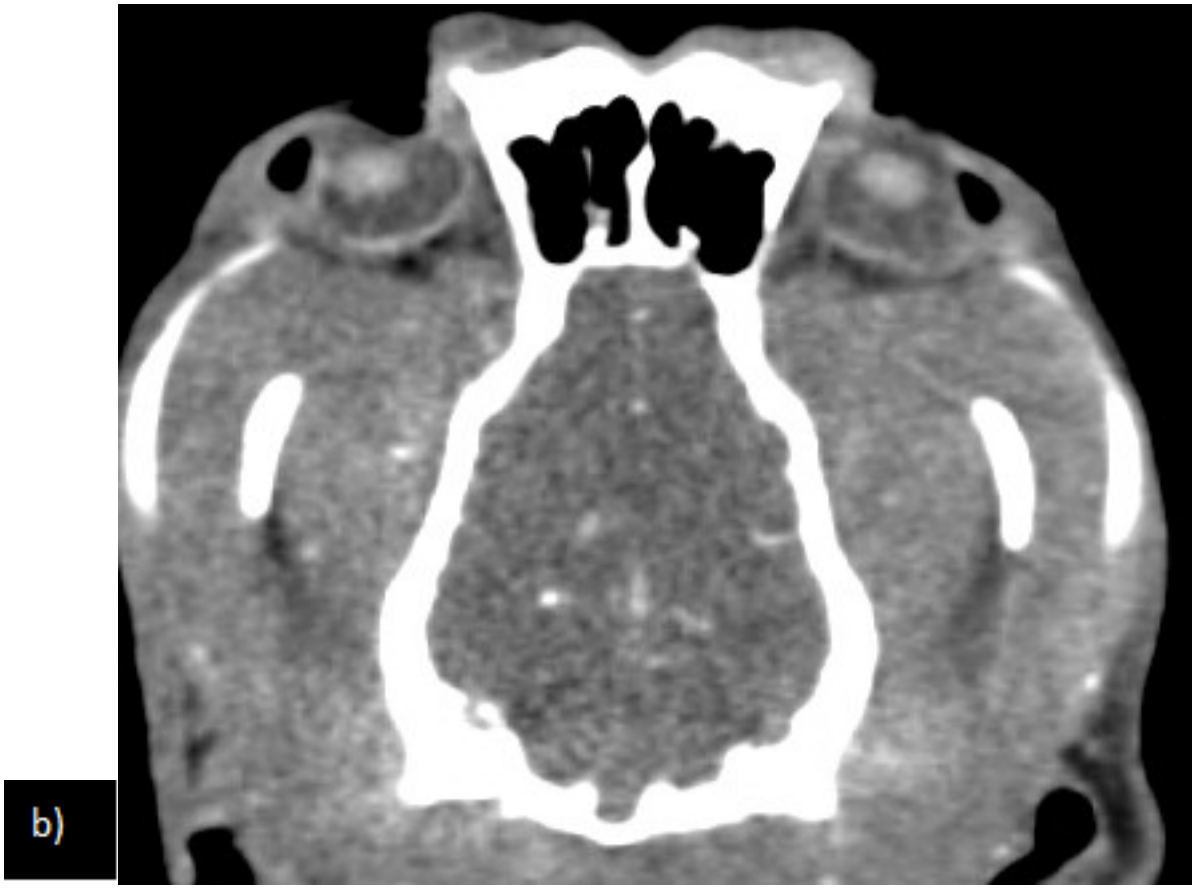


Figure 1: Arterial phase PMCTA image of a two-year-old male entire Staffordshire terrier: a) sagittal and b) dorsal plane multiplanar reformatted images. The images illustrate the extent and degree of opacification of contrast with clear contrast enhancement in the cerebral and facial arteries.

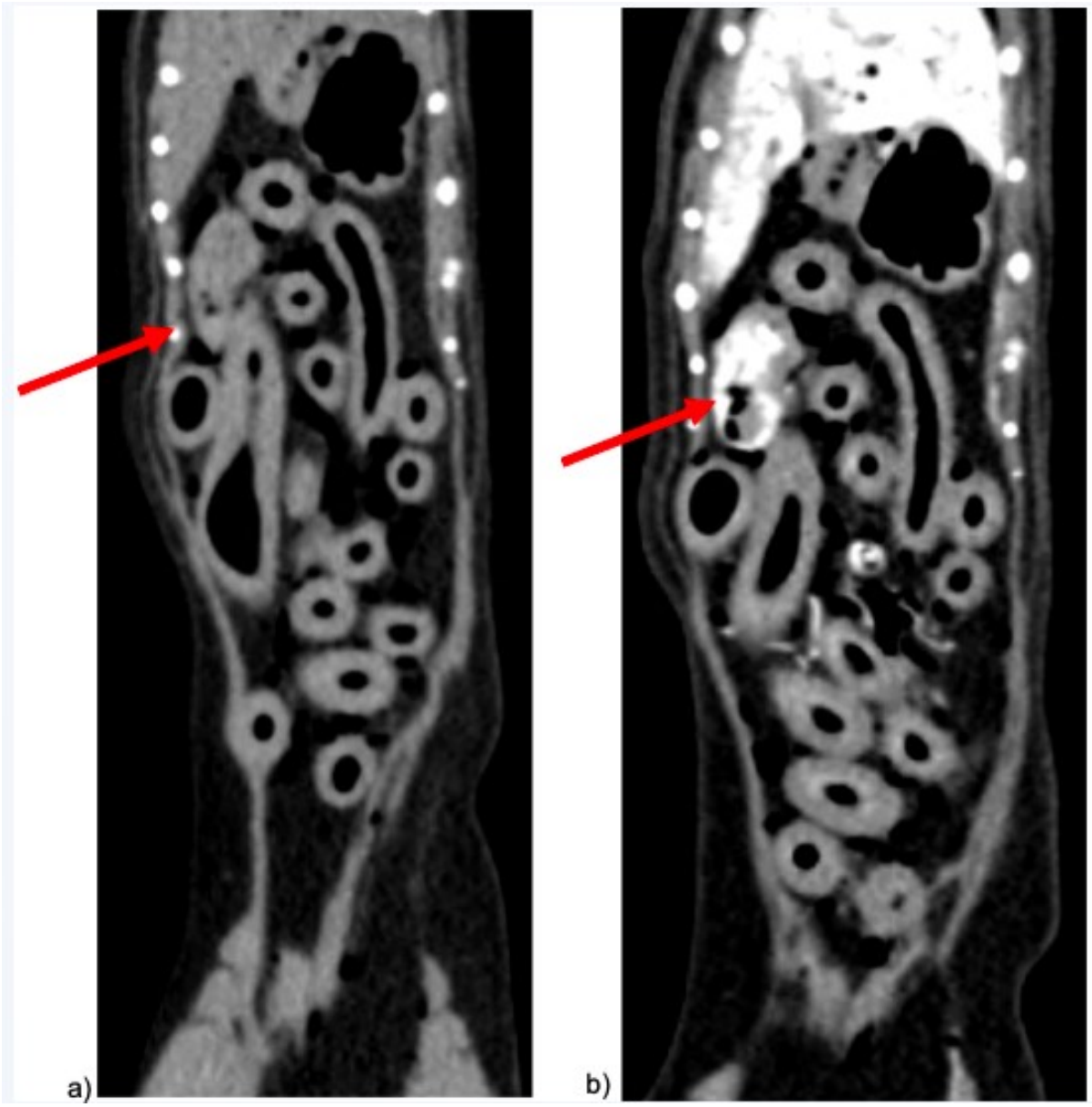
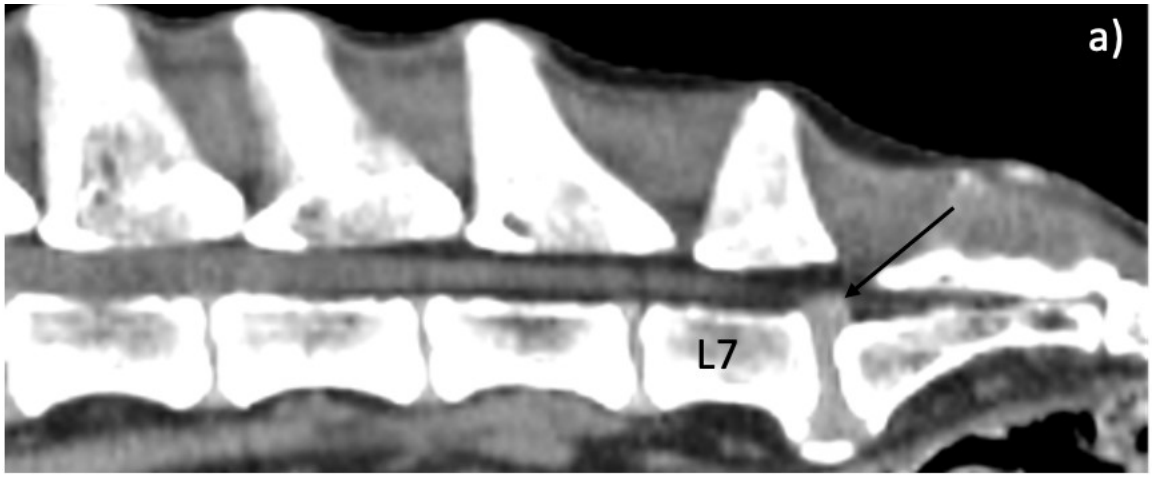


Figure 2: Both images are PMCT dorsal plane reformats of the abdomen of a 12-year-old domestic shorthair cat. Image a) is pre-contrast and image b) is in the arterial phase. The caecum is diffusely thickened and contrast-enhancing (red arrow). This lesion was thought to be lymphoma on PMCTA and found to be inflammatory typhlitis on histology.



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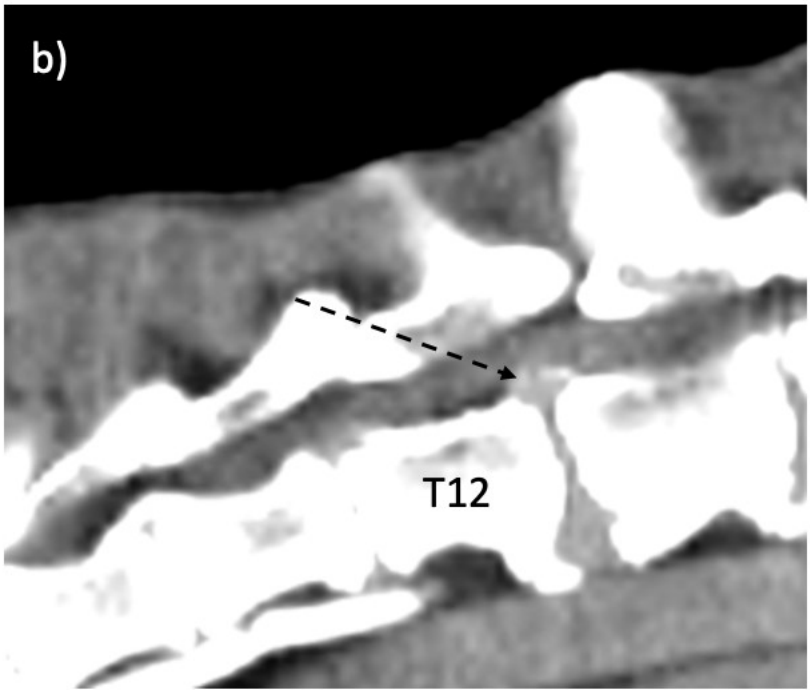

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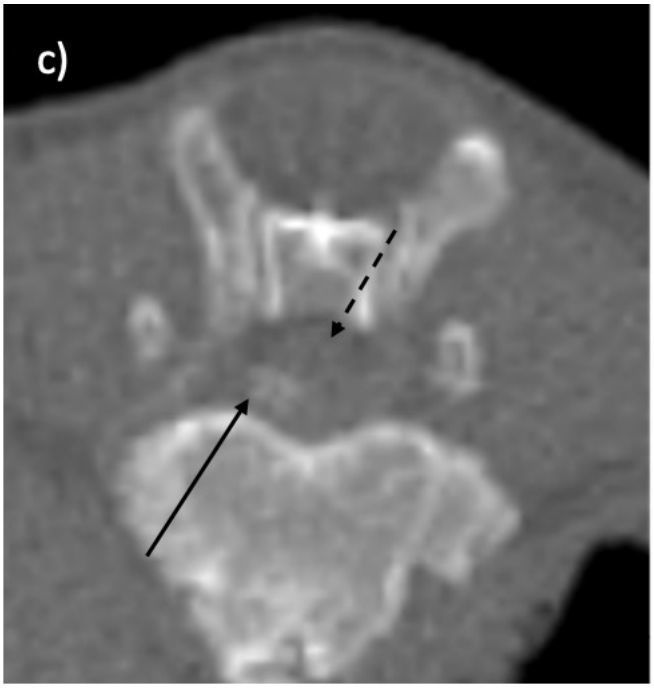

Figure 3: PMCT images of the lumbar and sacral spine in a 16-year-old male neutered dog. Soft tissue window sagittal plane reformat a) and b), and bone window transverse plane reformat at the level of T12-T13 disc space c).

Image a) shows intervertebral disc protrusion at L7-S1 (black arrow).

Image b) Extruded mineralised intervertebral disc material is present in the ventral aspect of the vertebral canal (dashed arrow) at T12-T13 and ventral spondylosis deformans (black arrow). Image c) Mixed mineral and soft tissue attenuating extruded disc material (black arrow) causing right ventrolateral compression of the spinal cord (dashed arrow).

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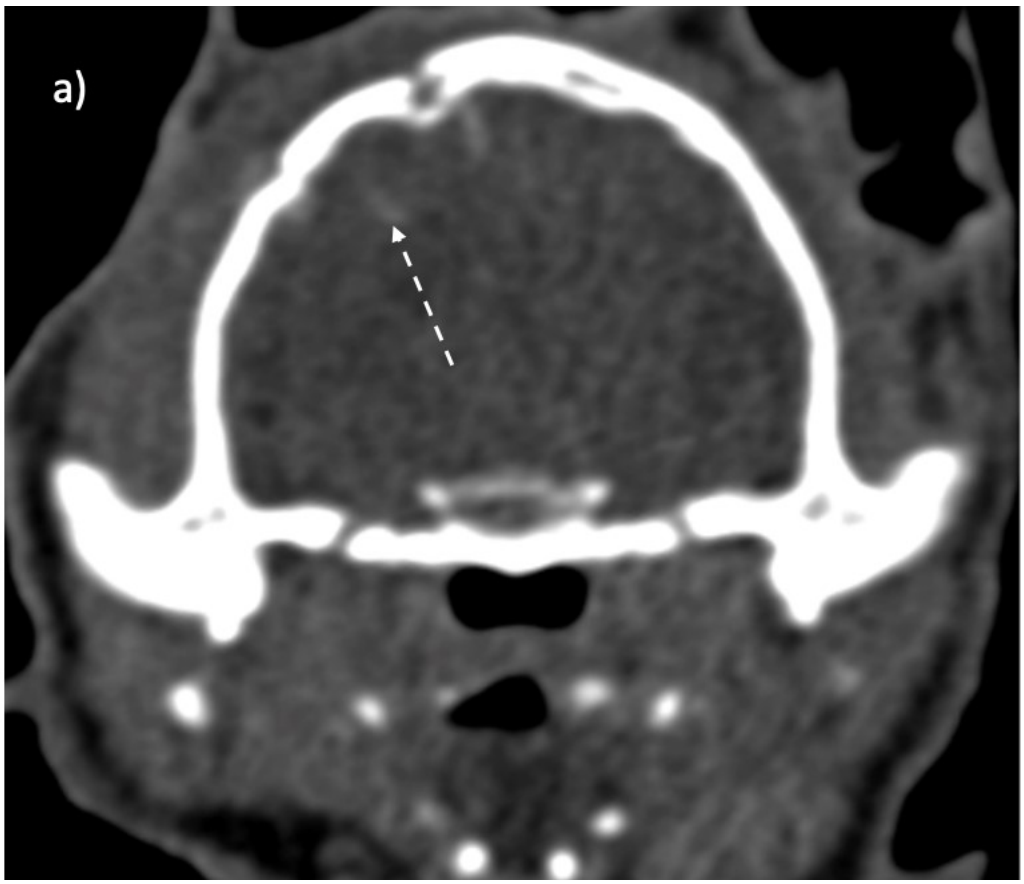

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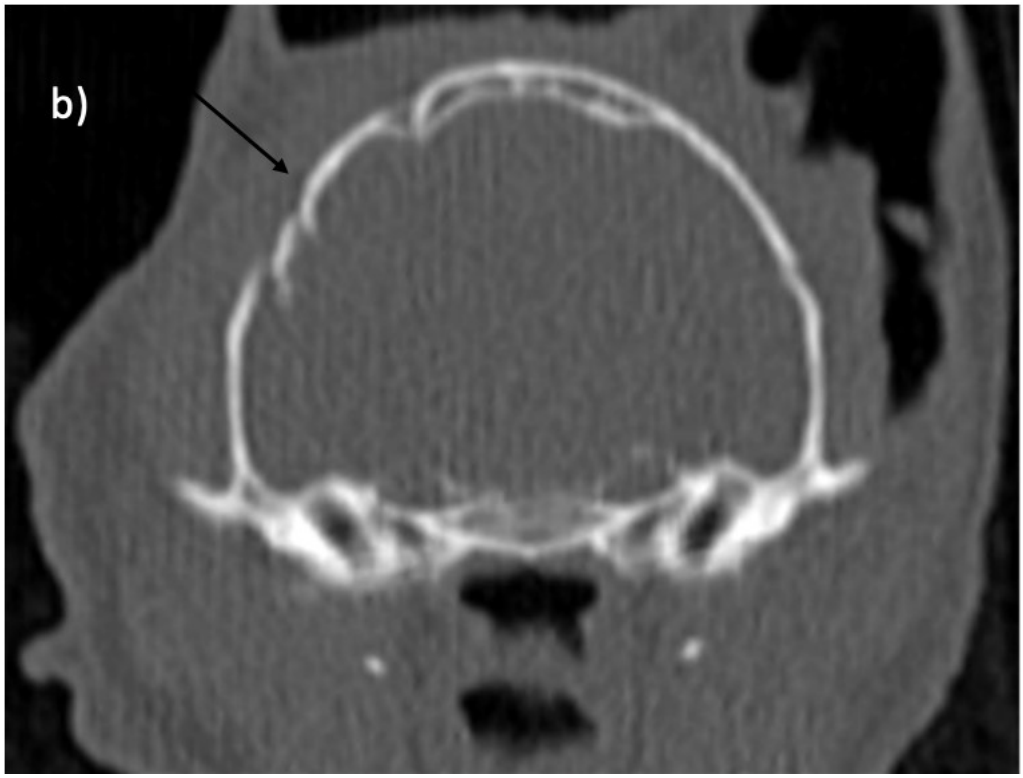

Figure 4: PMCTA images of the skull of an 11-year-old male neutered cat. Image a) is a soft tissue window transverse plane image in the venous phase. Image b) is a pre-contrast bone window transverse plane image. The skull fractures (black arrow) to the right temporal and parietal bones are best appreciated in the bone window. The soft tissue window shows ventroaxial to the fracture there is contrast enhancement of the meninges (dashed arrow).