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Abstract:	A six-year-old unilateral cryptorchid dog presented with episcleral haemorrhage, ecchymosis and pancytopaenia. Abdominal imaging confirmed the presence of a caudal abdominal mass suspected to be a Sertoli cell tumour with associated myelotoxicity. The presence of myelotoxicity induced thrombocytopaenia and the delay in obtaining serum oestradiol results can make it challenging to confirm hyperoestrogenism in suspected Sertoli cell tumours without overt feminisation. In these cases, risks of haemorrhage are high and preclude invasive sampling. Preputial cytology was used as a rapid and simple way to diagnose hyperoestrogenism in a dog without overt signs of feminisation and where the risk of significant haemorrhage precluded abdominal mass sampling. This technique can potentially detect hyperoestrogenism before clinical signs of feminisation and can readily be carried out in first opinion practice. A postmortem examination following sudden deterioration confirmed the presence of a Sertoli cell tumour in the retained testicle and intracranial haemorrhage secondary to thrombocytopaenia.

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TITLE OF CASE

Utility of preputial cytology to diagnose hyperoestrogenism in a dog with an intra-abdominal Sertoli cell tumour with concurrent thrombocytopaenia and intracranial haemorrhage

SUMMARY

A six-year-old unilateral cryptorchid dog presented with episcleral haemorrhage and ecchymosis. A complete blood count was consistent with pancytopaenia. Abdominal imaging confirmed the presence of a caudal abdominal mass suspected to be a Sertoli cell tumour with associated myelotoxicity. The presence of myelotoxicity induced thrombocytopaenia and the delay in obtaining serum oestradiol results can make it challenging to confirm hyperoestrogenism in suspected Sertoli cell tumours without overt feminisation. Preputial cytology was used as a rapid and simple way to diagnose hyperoestrogenism in a dog without overt signs of feminisation and where the risk of

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significant haemorrhage precluded abdominal mass sampling. This technique can potentially detect hyperoestrogenism before clinical signs of feminisation and can readily be carried out in first opinion practice. A postmortem examination following sudden deterioration confirmed the presence of a Sertoli cell tumour in the retained testicle and intracranial haemorrhage secondary to thrombocytopaenia.

BACKGROUND

Sertoli cell tumours (SCTs) are the most commonly reported tumours associated with abdominally retained testicles in dogs and can be a cause of hyperoestrogenism syndrome.[1] SCTs are hormonally active tumours originating from gonadostromal tissue within the testes and produce oestrogens and inhibin.[2] Cryptorchid dogs have a 13.6 times greater risk of developing testicular SCT or seminoma compared to entire male dogs with fully descended testicles, although metastasis of SCTs to regional lymph nodes, the liver and spleen is thought to be rare (2-14% of cases).[3-5] Seminomas are a type of testicular tumour that originates from germinal epithelium and they have rarely been reported to produce oestrogen with associated with feminisation.[6, 7] Non-functional seminomas and SCTs can be found concurrently.[8] Paraneoplastic hyperoestrogenism results in feminisation syndrome, reported in approximately 24-39% of dogs and common clinical signs include: gynaecomastia, symmetrical alopecia, penile atrophy, pendulous prepuce, linear preputial erythema and attraction to other male dogs.[5, 9]

Up to 57% of dogs with hyperoestrogenism are reported to develop overt signs of feminisation.[10] It has been hypothesized that it may be the oestrogen to testosterone ratio that results in the clinical consequences of feminisation rather than the absolute oestrogen concentration.[11]

Preputial cytology may have a greater clinical utility than serum oestradiol in demonstrating clinical consequences of the hormonal imbalance. Increased concentrations

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of oestradiol cause cornification of the preputial epithelial cells and the presence of more than 20% superficial squamous epithelial cells has been reported to have a high sensitivity (80%) and specificity (98%) for a serum oestradiol concentration >40 pmol/L (> 10.9 pg/mL), (RI, <25 pmol/L [RI, <6.8 pg/mL]).[12] The exact mechanism of mucosal epithelial cornification is unclear; it has been postulated that some areas of the skin involved in oestrogen-induced alopecia have higher expression of oestrogen receptors and this could also be valid for preputial cells. Although not common, oestrogen induced myelotoxicity (OIM) has been reported in association with SCTs.[13] The precise pathogenesis is poorly understood but potentially involves the production of a myelopoiesis inhibitory factor by thymus stromal cells.[14-16] The increased risk of Sertoli cell tumour development in cryptorchid dogs and consequences of oestrogen associated myelosuppression reinforces the importance of early surgery to remove these retained testicles in affected dogs.

CASE PRESENTATION

A 15.1 kg, six-year-old male entire whippet weighing 14.9 kg presented to the primary care veterinarian with a history of excessive bleeding as a result of traumatic detachment of the nail of the dew claw of the right fore limb. Prior clinical examination had identified the dog to be cryptorchid with only a small right testicle palpable within the scrotum. The nail injury was medically managed with a silver nitrate dressing and foot bandage; robenacoxib (2 mg/kg SC injection) and a seven day course of potentiated amoxicillin-clavulanate (16 mg/kg BID PO).[*,†] The dog became progressively hyporexic and at a follow up examination seven days after the initial presentation the referring veterinary surgeon reported a tense abdomen upon palpation and a quiet demeanour. A second injection of robenacoxib (2 mg/kg SC) was given on the left lateral cervical region and a three day

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course of robenacoxib (1.3 mg/kg PO SID)‡ was dispensed for continuation at home the following day.

Within a few hours of this consultation, the owner noted a progressive subcutaneous swelling develop at the site of the second robenacoxib injection and the dog re-presented 24 hours later with lethargy, bleeding from the recent SC injection site and haematochezia identified on rectal examination. A complete blood cell count with an in-house automated analyser revealed a moderate anaemia (HCT, 28%; RI, 37-61%) and severe neutropaenia (0.01 x 10^9/L; RI, 2.95-11.64 x 10^9/L and severe thrombocytopaenia (0 x 10^9/L; RI, 148-484 x 10^9/L). An abdominal ultrasound was carried out identifying a caudal abdominal mass and the dog was referred eleven days after the initial consultation for bleeding from the dew claw on the right fore limb.

On presentation, the dog was quiet, alert and responsive with a body condition score of 4/9. Bilateral episcleral haemorrhage was present along with inguinal and preputial ecchymoses. A subcutaneous swelling approximately 10 cm x 10 cm was seen on the left lateral side of the neck with a central puncture wound. The previously damaged dew claw nail of the right fore limb was identified along with reportedly progressive mild, diffuse subcutaneous swelling extending distally from the level of the carpus. A single, small testicle was identified within the scrotum. The dog was cardiovascularly stable and the rest of the clinical examination was unremarkable.

INVESTIGATIONS

A complete blood count with blood smear review confirmed a mild, non-regenerative, normocytic normochromic anaemia (HCT, 31%; RI, 39-55%) and automated reticulocyte count of 6.2 x 10^9/L (RI, 0-60 x 10^9/L), a severe neutropaenia (0.08 x 10^9/L; RI, 3.6-12 x 10^9/L) with few band neutrophils and mild toxic changes (Döhle bodies), and a severe

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thrombocytopaenia (2 x 10^9/L (RI, 200-500 x 10^9/L) with less than 1 medium sized platelet per high power field and no evident clumping. There were no macroplatelets visible on the blood smears reviewed. Packed cell volume (PCV, 31%; RI, 39-55%) and plasma total solids (TS_{plasma}, 58 g/L; RI, 60-80 g/L [0.58 g/dL; RI, 0.60-0.80 g/dL] were also measured. Serum biochemistry revealed a mild hypoproteinaemia (50.4 g/L; RI, 58-73 g/L [0.5g/dL; RI, 0.58-0.73 g/dL]) due to mild hypoalbuminaemia (23.8 g/L; RI, 26-35 g/L [0.24 g/dl; RI, 0.26-0.35 g/dl]), other findings were unremarkable. Prothrombin (PT) time (11 s; RI, 11-17 s) and activated partial thromboplastin time (aPTT) (75 s; RI, 72-102 s) were within reference ranges.

A serum sample was submitted for quantification of oestradiol-17☐, which revealed a markedly elevated serum oestradiol-17☐ concentration at 722 pmol/L; RI, < 10 pmol/L (196.7 pg/mL; RI, <2.72 pg/mL).

The dog was blood typed as weak DEA 1 positive.[±] Urinalysis on a free catch sample and analysed with an automated urine sediment and dipstick analyser[§] identified: visually clear, yellow, hyposthenuric (USG 1.005) urine with 250 RBCs/μL indicating mild haematuria. Three-view thoracic radiographs were within normal limits. An abdominal radiographic study identified an ovoid mass with defined margins within the caudal abdomen, cranial to the apex of the urinary bladder (Figure 1A). An abdominal ultrasound identified the presence of a heterogenous mass within the caudal abdomen measuring 4.3 cm x 2.3 cm, located caudal to the left kidney (Figure 1B). There was no evidence of regional lymph node enlargement. A single, small testicle (2 cm x 0.8 cm) was present in the scrotum with a normal echogenic appearance.

Two preputial swabs were obtained using a fine-tipped sterile cotton swab☐ which was carefully inserted and rotated ten times along the medial mucosal aspect of each side of the

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prepuce adjacent to the penis. Several cytological smears were prepared from each swab. The slides were stained using May-Grunwald Giemsa stain. Cytological evaluation was carried out by a board certified veterinary clinical pathologist. Cytology revealed the presence of approximately 85% superficial epithelial cells and 15% intermediate epithelial cells (Figure 1C). A moderate mixed population of bacteria (cocci and bacilli) with no evidence of inflammation was also present. Preputial cytology of a healthy dog is provided for comparison (Figure 1D).

DIFFERENTIAL DIAGNOSIS

The main differential diagnosis for the above clinical and diagnostic findings was a testicular hormone secreting tumour; primarily SCT with associated hyperoestrogenism and secondary OIM. Other less likely differentials included intra-abdominal testicular torsion, other neoplasm with associated bone marrow involvement (e.g., leukaemia/lymphoma) or an abscess.

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TREATMENT

Given the neutropaenia and increased risk of developing a nosocomial infection, the dog was barrier nursed (handled with gloves and gown) and prophylactically administered potentiated amoxicillin-clavulanate (20 mg/kg, IV, q 8 h).[¥] Given numerous, prior mild cutaneous hypersensitivity reactions observed in dogs following the administration of potentiated amoxicillin-clavulanate within the author's hospital, chlorphenamine[δ] (4 mg, IV, q 8 h) was administered prophylactically prior to each dose according to hospital guidelines. Tranexamic acid (15 mg/kg, IV, q 8 h)[κ] was started in an attempt to augment clot formation and minimize ongoing bleeding, given the potential for breed related hyperfibrinolysis alongside the severe thrombocytopaenia.

Although not without risk of considerable (potentially uncontrollable) bleeding, an exploratory coeliotomy with the aim of surgical removal of the suspected SCT was scheduled with administration of a whole blood transfusion peri-operatively to mitigate the effects of intra-operative bleeding.

OUTCOME AND FOLLOW-UP

Within 24 hours following admission, the dog experienced an acute deterioration in mentation, becoming obtunded with a Modified Glasgow Coma Score (MGCS) of 15/18 and displayed proprioceptive ataxia. A systolic blood pressure (measured with a Doppler blood pressure unit) of 195 mmHg and a heart rate of 60 bpm was recorded. An increase in intracranial pressure secondary to an intracranial haemorrhage as a result of thrombocytopaenia was strongly suspected and mannitol (0.5 g/kg slow IV) was administered.[$] This resulted in a mild improvement in mentation. A repeat PCV and TS identified a decreased PCV (17%; RI, 39-55%) and TS (50 g/L; RI, 60-80 g/L [0.5 g/dL; RI, 0.60-0.80 g/dL]) and no evidence of external or intra-thoracic and abdominal

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haemorrhage based on a focused sonographic assessment. Given the acute deterioration, surgical intervention was aborted and the dog was euthanised.

A post-mortem examination was performed. The necropsy identified moderate amounts of haemorrhage throughout the subdural and subarachnoid spaces, particularly overlying the right caudal occipital lobe and dorsal cerebellum, alongside bilateral blood pooling within the ventral temporal fossa (Figures 2A and 2B). There was no macroscopic or microscopic evidence of cerebellar herniation or intra-cerebral/intra-cerebellar haemorrhage. Histology of the left cryptorchid testicle identified a pseudoencapsulated, expansile proliferation of polygonal to columnar cells which formed packets and larger islands supported by extensive fibrovascular stroma with morphological features consistent with a SCT (Figure 2C). Alongside this proliferation, within an area of remnant atrophic seminiferous tubules there was an expansile proliferation of polygonal cells forming sheets within tubules which were supported by minimal stroma, with morphological features consistent with a seminoma. The right scrotal testicle had chronic seminiferous tubular atrophy, whilst the prostate gland had severe diffuse squamous metaplasia, a common consequence of excessive exposure to oestrogens (Figure 2D).[1] The bone marrow was diffusely hypocellular, which interpreted alongside the clinically documented pancytopaenia, was likely as a result of myelotoxicity induced by hyperoestrogenism.

DISCUSSION

This case report describes the value of preputial cytology as a simple, quick and non-invasive method to diagnose hyperoestrogenism as a result of an abdominal SCT in a dog without overt clinical signs of feminisation, with concurrent profound thrombocytopaenia and non-traumatic subcutaneous and intracranial haemorrhage.

The cytological findings were interpreted in relation to a previous study, where the presence of more than 20% of superficial cells in preputial cytology was associated with a

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serum oestradiol-17□ concentration > 40 pmol/L; RI, < 25 pmol/L (>10.9 pg/mL; RI, <6.8 pg/mL).[12] This was consistent with the presence of hyperoestrogenism and further supported the presumed diagnosis of SCT and associated OIM.

Preputial cytology is a good diagnostic method to identify hyperoestrogenism without overt signs of feminisation. It is possible that a positive preputial cytology result is an early indicator of hyperoestrogenism with SCT, however, future studies investigating the underlying pathophysiology and the reliability of this method in this subset of cases are necessary.

Surgical excision of SCTs remains the treatment of choice and the presence of OIM is associated with a guarded prognosis, especially given the slow bone marrow recovery and the potential life threatening complications associated with the secondary pancytopaenia. Where surgery is successful, it can take months for bone marrow to recover from the effects of hyperoestrogenism, with some reports of incomplete recovery.[15, 17]

The presence of profound thrombocytopaenia and evidence of active haemorrhage in this case posed some diagnostic challenges and it was deemed unsafe to carry out fine needle aspirates or biopsies of the abdominal mass to confirm a diagnosis. Serum oestradiol-17□ concentrations can be measured, however due to this being performed by an external laboratory□, it was anticipated there would be a delay of several days before results were available and a value within range is not sensitive enough to exclude bone marrow suppression.[18-20] The serum oestradiol results in this case were obtained post euthanasia. Alternatively, measuring the serum testosterone levels in this case and determining the oestrogen to testosterone ratio would have been an interesting additional parameter; however measuring multiple sexual hormones is expensive, requires specific laboratories and mildly altered values may be difficult to interpret.

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A bone marrow biopsy can be performed to confirm the presence of bone marrow hypoplasia or aplastic anaemia, although histopathologic findings are not pathognomonic of OIM. The preputial cytology in conjunction with the other diagnostic findings in this case were sufficient to provide a diagnosis of SCT and hyperoestrogenism and therefore a bone marrow biopsy was deemed unnecessary.

Platelet concentrate products were not available for transfusion and given the rapid deterioration as a result of intracranial haemorrhage, it was not possible to consider other treatment modalities. Lithium carbonate has been used with some success for oestrogen induced bone marrow hypoplasia in dogs to stimulate the division of pluripotent stem cells; although this is typically considered as part of chronic management once the source of hyperoestrogenism has been removed.[21, 22]

It is possible that the breed in this case may have potentiated the effects of the thrombocytopaenia. Several studies have proposed hyperfibrinolysis as a potential mechanism for excessive bleeding in sighthounds after minor trauma or routine surgery; although the exact mechanism still remains unclear.[23, 24] This was a factor taken into consideration with this case with the administration of tranexamic acid[κ] from the point of admission.

Recent studies using viscoelastic assays have identified a number of haemostatic differences in Greyhounds compared to other breeds. [25, 26] However, factors such as elevated PCV and variable test sensitivity may affect such conclusions and further research into this area is warranted.[27] Previous reports of abdominal SCTs in dogs have documented profound thrombocytopaenia without associated intracranial haemorrhage.[28]

The use of thromboelastography in this case may have been useful in further quantifying

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the risk of bleeding in this case, although, this was clinically evident based on the existing presence of ecchymosis and episceral haemorrhage.

LEARNING POINTS/TAKE HOME MESSAGES

- Thrombocytopaenia in dogs with Sertoli cell tumours and oestrogen induced myelotoxicity mean that invasive diagnostic techniques risk significant haemorrhage.
- Preputial cytology proved to be a highly useful and simple diagnostic technique in identifying changes to the mucosal epithelium consistent with feminisation.
- It is hoped that this case report will encourage the use of this non-invasive and rapid diagnostic technique in similar cases and further the investigation of the relationship between oestradiol-17□, preputial epithelial cytology and oestrogen induced myelotoxicity.

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Footnotes

□Idexx Procyte DX Haematology Analyser, Wetherby, UK.

□Amies charcoal cotton mini tip swab, Technical Service Consultants Ltd., Lancashire, UK

*Onsior 20mg/ml solution for injection for cats and dogs, Elanco, Hook, UK.

†Synulox 250 mg palatable tablets, Zoetis, Surrey, UK.

‡Onsior 20mg tablet for dogs, Zoetis, Surrey, UK.

§Idexx SediVue DX and VetLab UA Analyser, Wetherby, UK.

□NationWide Specialist Laboratories, Cambridge, UK.

¥Augmentin, GlaxoSmithKline, Middlesex, UK.

δChlorphenamine Maleate, Archimedes Pharma, Reading, UK.

κCyklokapron 100mg/ml, Pfizer, Sandwich, UK.

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[±]Alvedia canine blood typing (immunochromatography), Limonest, France.

[$]Mannitol 10% intravenous solution, Fresenius Kabi, Toronto, Canada.

The authors have no conflicts of interest to declare.

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FIGURE/VIDEO CAPTIONS

Figure 1: (A) Abdominal radiograph showing the circular outline of the testicular mass (arrow). (B) Ultrasound image of the testicular mass. (C) Normal preputial cytology, note the presence of intermediate epithelial cells and non-degenerate neutrophils (similar to vaginal smear of bitches in anoestrus), May-Grünwald-Giemsa, 20 x. (D) Preputial cytology from the patient, with hyperoestrogenism secondary to SCT; the vast majority are superficial cells with few bacteria in the background and no inflammation (similar to vaginal smear of bitches in oestrus), May-Grünwald-Giemsa, 20 x. Permission for the use of these images has been granted by the owner of the dog in this case report.

Figure 2: Representative images of pathological lesions. (A) Extensive subdural and subarachnoid haemorrhage, particularly over the occipital lobes. (B) Atrophic right testicle (left of the image) and the neoplastic left testicle (right of the image) expanded by a locally extensive, white to tan, firm mass (SCT) with multifocal haemorrhage. (C) Low power (x25) and high power (x400) magnification of the SCT, with proliferation of columnar cells supported by extensive stroma, Haematoxylin and Eosin. (D) Extensive prostatic squamous metaplasia, Haematoxylin and Eosin. Permission for the use of these images has been granted by the owner of the dog in this case report and the clinical pathology team involved.

OWNER'S PERSPECTIVE

N/A

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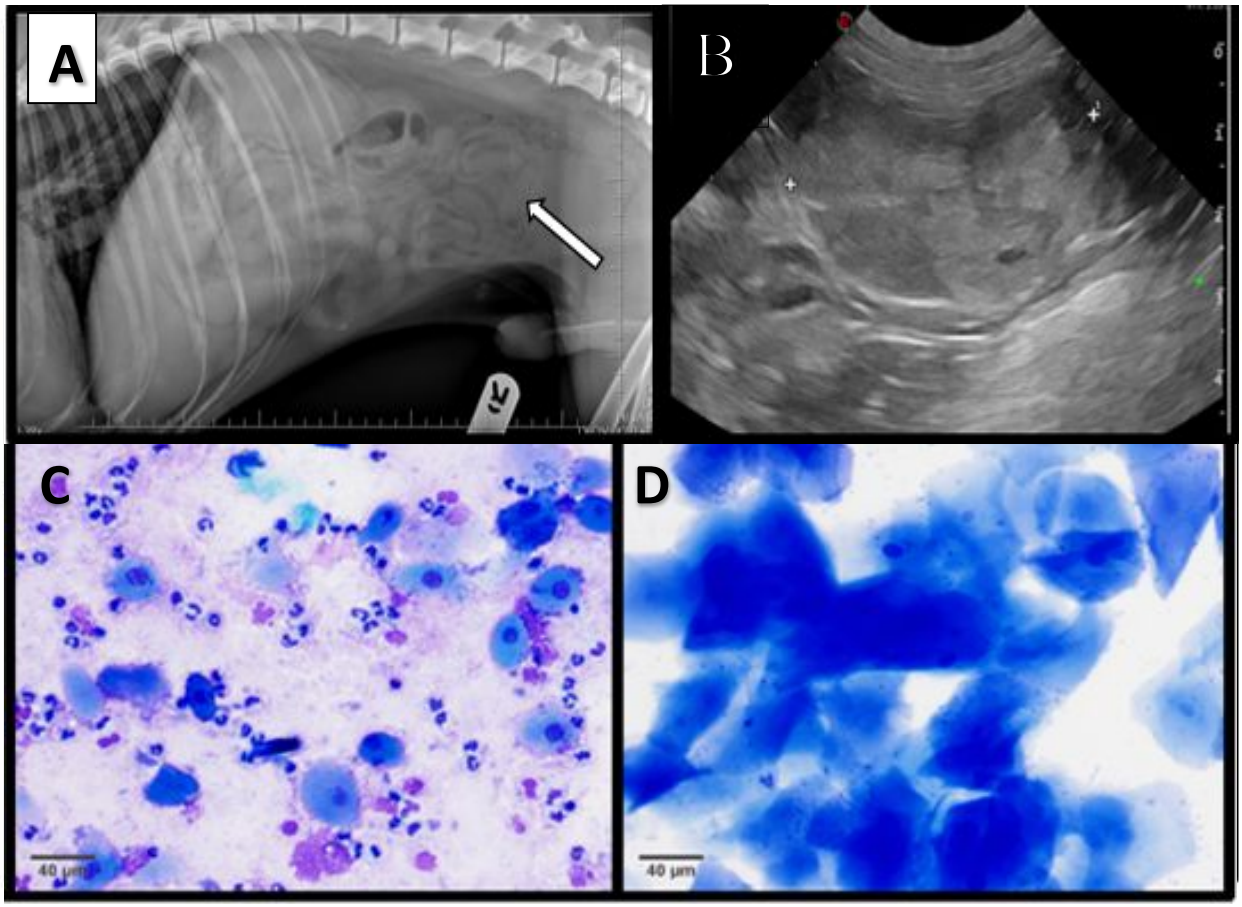

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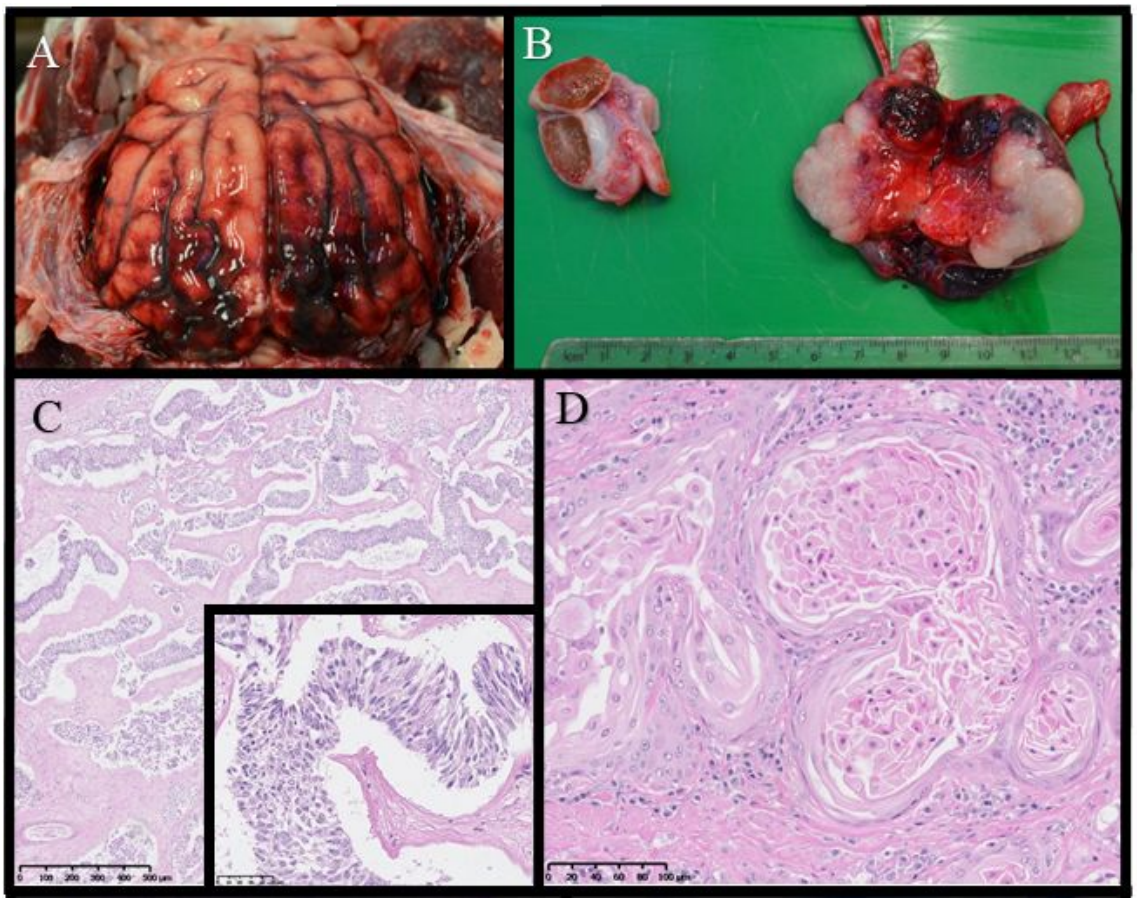

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