Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2018-2019

C o n f e r e n c e 20

20 March 2019

Conference Moderator:

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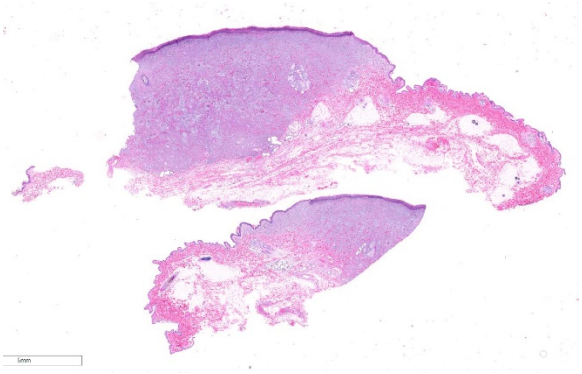
CASE I: D12-49 (JPC 4041970).

Signalment: 8-year-old, male, castrated Labrador retriever (*Canis lupus familiaris*)

History: A cutaneous mass adjacent to the prepuce was surgically removed with wide margins and submitted for histologic evaluation. Following excision, the mass recurred and continued to grow despite treatment with prednisone and vinblastine. Therapy was switched to Palladia and resulted in partial remission for one month, at which point the neoplasm began to increase in size again. Euthanasia was elected due to poor prognosis and failed response to therapy.

Gross Pathology: None available.
Laboratory results: None available
Microscopic Description:
Expanding the dermis and elevating the overlying ulcerated epidermis is a well circumscribed, unencapsulated neoplasm consisting of numerous mast cells with marked anisocytosis and anisokaryosis, karyomegaly, bizarre nuclei, no apparent granules, 1-2 irregularly round nuclei, and a mitotic index of 5-6 per high power field, with bizarre mitotic figures. These cells are admixed with high numbers of eosinophils



Haired skin, dog: Sections of a dermal mast cell tumor with an ulcerated surface was submitted for examination. (HE, 5X)

and multifocal collagenolysis (flame figures). The margins appear complete.

Contributor's Morphologic Diagnoses: Haired skin: Mast cell tumor, high grade.

Contributor's Comment: Mast cell tumors account for 15-20% of skin tumors in dogs, and are the most frequent malignant tumor of the skin. In 2011, Kiupel et al. proposed a two-tier histologic grading system to more accurately predict the biological behavior of canine cutaneous mast cell tumors.[2] This two-tier approach replaced the commonly used three-tier Patnaik system, and has since been shown to provide useful prognostic information with increased consistency of grading among veterinary pathologists.[1,6].

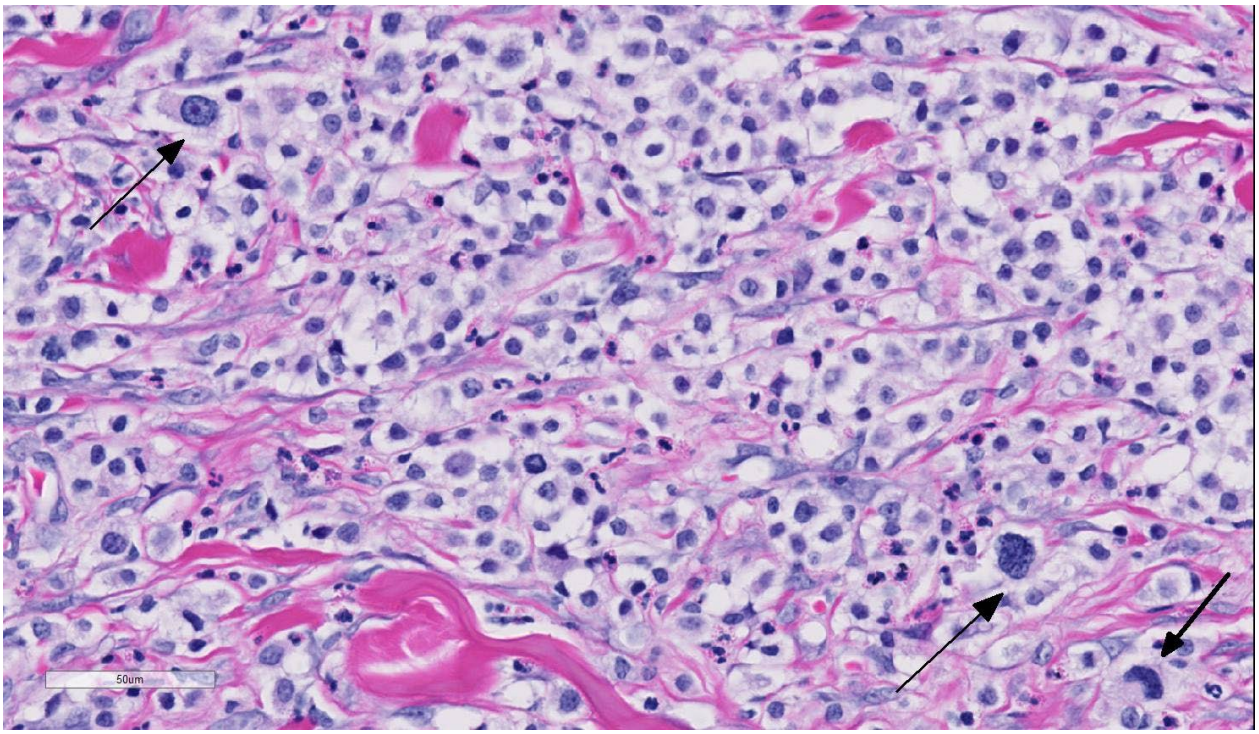
This particular neoplasm has a very high mitotic rate with marked atypia of neoplastic cells, and serves as a prime example of a high grade neoplasm. Clinical follow-up on this patient revealed recurrence of the neoplasm at the site of excision and failure of response to treatment.

While not present in this case, it was recently reported that canine cutaneous mast cell tumors can exhibit epitheliotropism, and should be included as a differential for cutaneous round cell neoplasms with epitheliotropic behavior.[4]

Contributing Institution:
Tri-Service Research Laboratory
JBSA Ft. Sam Houston, TX

JPC Diagnosis: Haired skin: Mast cell tumor, high grade.

JPC Comment: In 2011, a 28-pathologist, 16-institution study on histologic grading of cutaneous mast cell tumors was published

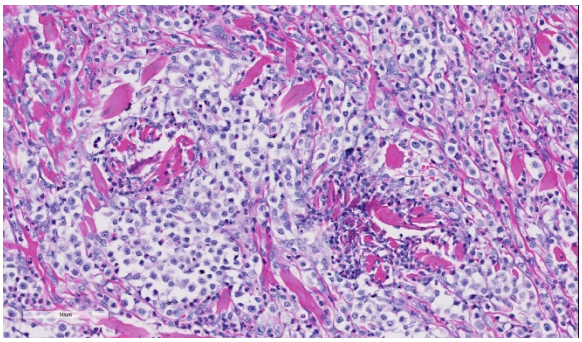


Haired skin, dog: Neoplastic cells are poorly granulated, and cells with pleomorphic nuclei exceed 3 /2.37mm2 fields (arrows) (HE, 400X)

which has significantly increased concordance between pathologists in the grading and prognosis of canine mast cell tumors.[2] This system is currently in use at the Joint Pathology Center and in most diagnostic laboratories. The study replaced previous 3-tier studies by Bostock (1973) and Patnaik (1984), both 3-tier studies in which interobserver variation resulted in an skewing of grading toward intermediate (grade 2) grades.[2]

The current two-tier system classifies cutaneous mast cell tumors into low- versus high-grade. The diagnosis of high grade mast cell tumors is based on the presence of ANY ONE of the following criteria: seven mitotic figures per ten 2.37mm^2 fields, 3 multinucleated cells or cells with bizarre nuclei per ten 2.37mm^2 fields, or karyomegaly (with nuclei of at least ten per cent of neoplastic cells varying by at least two fold. In this particular case, the participants were in agreement with an elevated mitotic count as well as the presence of an increased number of cells with bizarre nuclei.[2] The count of mitotic figures should be performed in the areas of the slide with the highest frequency of mitotic figures.[2]

The kit protein is important in the proliferation, differentiation, migration and survival of mast cells. A variety of c-kit mutations have been identified in mast cell tumors, with the most common mutation (in exon 11) being associated with a significantly worse prognosis.[3] In a study, 19/49 dogs with cutaneous mast cell tumors possessing a c-Kit mutation on exon 11 died within a year due to MCT-associated disease. Mutations on chromosome 8 and 9 compose less than 5% of overall mutations each, but have no impact on prognosis. PCR testing on this neoplasm was performed at the Michigan State Diagnostic Lab and was positive an activation duplication mutation in exon 11 of c-Kit, but was negative for exon 8 mutations.[3]



Haired skin, dog: Areas of collagen degradation are commonly seen in a number, but not all MCTs. (HE, 400X)

While mast cell tumors may arise in any organ, the skin is the most common site for mast cell tumor development in the dog. Another classification is important in evaluating cutaneous mast cells – that of a cutaneous versus subcutaneous location. Under previous grading systems, tumor depth was considered an adverse prognostic factor. Thompson et al. in 2011[5] evaluated followup data for 206 subcutaneous mast cell tumors (those restricted to the subcutaneous fat) and found that 6-month, 1-, 2- and 5-year survival times were 95%, 93%, 92%, and 86% respectively, indicating a significantly better prognosis for this subset of tumors. Only 4% exhibited metastasis, and 6% had local recurrence, although 56% of cases had incomplete tumor margins.[5]

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System for Canine Cutaneous Mast Cell Tumors to More Accurately Predict Biological Behavior. *Vet Pathol*. 2011;48(1):147-155.

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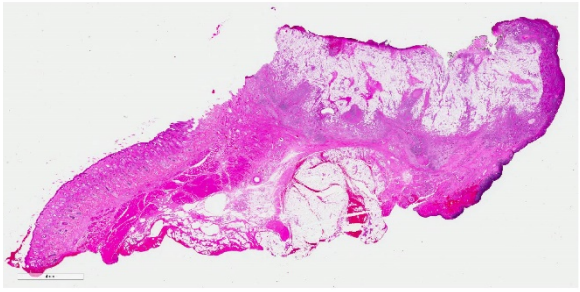
CASE II: SP-18-0001846 (JPC 4117533).

Signalment: 15-year-old, intact female, miniature horse, *Equus ferus caballus*, equine

History: This animal had a six month history of irritation of the right eye associated with a non-healing wound on the right lower eyelid. Physical examination revealed a 3cm in diameter, ovoid, ulcerated mass on the right eye lower eyelid that exuded yellow discharge.

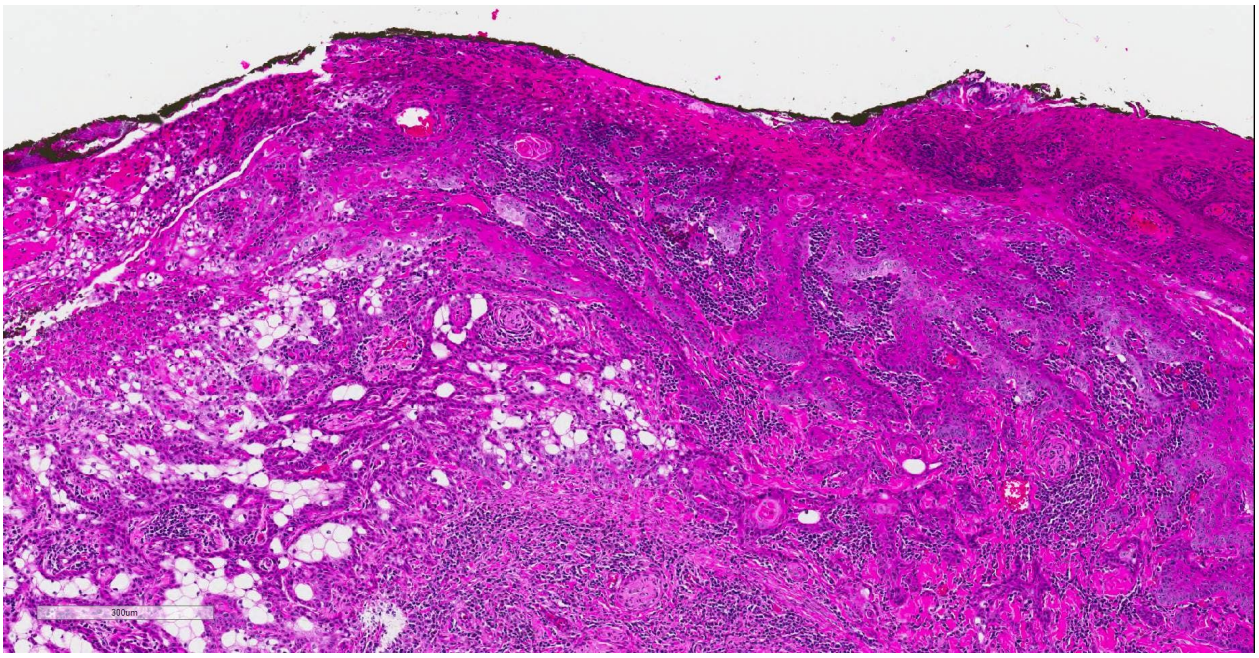
Gross Pathology: N/A

Laboratory results: N/A



Eyelid, horse. A section of ulcerated eyelid is submitted for examination. (HE, 4X)

Microscopic Description:
Histopathologic examination revealed a fairly well-demarcated, ulcerated dermal mass composed of neoplastic squamous epithelial cells arranged in anastomosing cords and nests that extended from the surface into the underlying dermis. Neoplastic cords near the surface and along the lateral margins of the mass had multifocal central keratinization. Neoplastic squamous epithelial cells merged with dense sheets of vacuolated polygonal cells towards the center of the mass. The center of the mass was also characterized by nests and cords of neoplastic cells composed of 1-3 outer layers of neoplastic squamous cells that were non-vacuolated and basaloid and rimmed central areas composed entirely of similar large vacuolated polygonal cells that formed sheets in the center of the mass. The non-vacuolated basaloid neoplastic squamous cells were round, contained a small to moderate amount of eosinophilic cytoplasm, and had round to ovoid, finely stippled nuclei that generally contained a single, large, central nucleolus. Anisokaryosis was moderate to marked, depending on the area, and there were occasional binucleated cells. There were 0-2 mitoses per high powered field. The vacuolated polygonal cells often had larger finely stippled nuclei that were peripheralized. Multifocally, nests and cords of neoplastic squamous epithelial cells invaded the underlying dense collagenous connective tissue, focally extended into the



Eyelid, horse. Anastamosing cords of squamous epithelium descend from the overlying epidermis, and at left are separated by polygonal clear cells. Numerous aggregates of lymphocytes, plasma cells, and fewer macrophages, eosinophilis and neutrophils are scattered throughout the neoplasm. (HE, 123X)

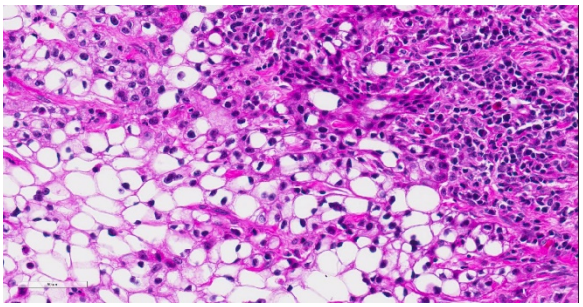
underlying skeletal muscle, and often surrounded nerves. In addition, there were multifocal perivascular and perineural inflammatory infiltrates comprised of moderate to large numbers of lymphocytes and plasma cells. In some regions, there was sebaceous gland hyperplasia along the surface of the haired skin. The dermis along the lateral margins of the neoplasm also had evidence of solar elastosis. Within the superficial dermis, there were foci of smudged degenerate collagen admixed with tangles of thickened, curled, lightly basophilic fibers that stained black with Verhoeff-van Gieson, consistent with elastin.

Immunohistochemistry for E-cadherin showed strong perimembranous labeling of neoplastic squamous, basaloid and vacuolated polygonal cells. Special staining with oil-red-O, periodic acid-Schiff (PAS), and mucicarmine did not reveal positive material within the cytoplasm of vacuolated polygonal cells.

Contributor's Morphologic Diagnoses: Clear cell squamous cell carcinoma

Contributor's Comment: While this neoplasm was organized in varying patterns, including extensive sheets of vacuolated cells, there was overt squamous differentiation in several areas. These histologic findings, in conjunction with the immunohistochemical and histochemical staining results, were consistent with a clear cell squamous cell carcinoma (SCC). There was no true tubular or acinar formation and vacuolated polygonal cells lacked cytoplasmic lipid, glycogen, and mucus, further excluding neoplasms of sebaceous or adnexal origin.[6-8,10,14,15]

To our knowledge, clear cell SCC has not been reported in animals. Variants of squamous cell carcinoma with clear cells have been described in dogs, but have not met the criteria of clear cell squamous cell carcinoma. A signet-ring squamous cell carcinoma was described in the scrotum of



Eyelid horse. Higher magnification of the transition between cords of squamous epithelium at right and clear cell squamous epithelium at left. (HE, 400)

one dog, but ultrastructurally clear cells contained intracytoplasmic lipid vacuoles and scattered glycogen.[6] The uncommon canine clear cell adnexal carcinomas are also negative for oil red O, but in contrast to our case, positive for PAS.[15] Other cutaneous neoplasms with clear cells that have been reported in dogs includes clear cell basal carcinoma, clear cell hidradenocarcinomas, sebaceous carcinomas, balloon cell melanomas, and liposarcomas.[7-8,15] None of these entities is characterized by squamous differentiation, and the clear cells in adnexal neoplasms are PAS positive. In horses, clear cell differentiation has only been reported in cutaneous basal cell tumors; however, those tumors lacked the squamous differentiation observed in our case.[14]

In humans, clear cell SCC is a rare neoplastic entity in the skin also referred to as hydropic SCC. The clear cell appearance is caused by hydropic degeneration of neoplastic squamous cells causing accumulation of intracellular fluid and not glycogen, lipid, or mucin.[9,13] Clear cell SCC is divided into three histologic types: keratinizing (type I), nonkeratinizing (type II), and pleomorphic (type III). The keratinizing, or type I, clear cell SCC, is defined by sheets or islands of clear neoplastic cells with peripherally displaced nuclei that are often indistinguishable from adipocytes, as well as some cells that have more vacuolated cytoplasm and resemble sebaceous cells.[10] Additionally, there are foci of keratinization or keratin pearl formation. Type II is characterized by anastomosing cords of neoplastic squamous cells with dense lymphoplasmacytic infiltrates and no keratinization, while type III demonstrates marked pleomorphism, vascular and perineural invasion, foci of squamous differentiation, and microcysts with acantholytic neoplastic cells. In all three types, there is no glycogen or mucin accumulation within clear cells, thus, clear cells are negative for PAS, mucicarmine, and alcian blue stains. AE1/AE3 and cytokeratin 7 have been used to confirm epithelial origin of the neoplastic clear cells.[9,11] The case presented here is most consistent with a type I clear cell squamous cell carcinoma.

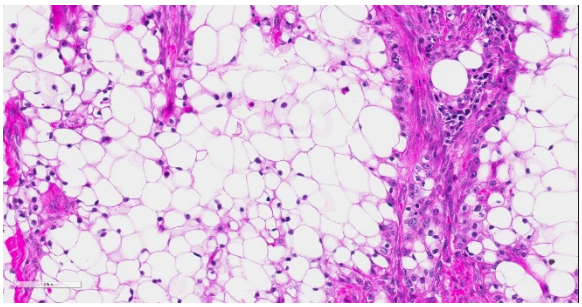
Squamous cell carcinomas are the most common neoplasm of the equine eye and adnexa.[5] SCC of the eyelid typically has an aggressive, locally invasive behavior, and carries a poor prognosis.[16] Metastasis is uncommon but may occur late in the course of disease and most often to the regional lymph nodes. We speculate that clear cell SCC will exhibit similar behavior, although this cannot be confirmed due to lack of follow-up in our case. The prognosis of this entity in humans is also unclear due to the scarcity of case reports. Of seven cases of clear cell SCC reported in humans, all but one have occurred on the head or neck of elderly white males who worked outdoors. One case was reported in a dark-skinned adult male who also worked outdoors. Of these reported cases, one patient died of metastatic disease, one died post-operatively, and one had recurrence after 3 months.[1,13]

In both humans and animals, chronic ultraviolet (UV) light exposure plays an important etiologic role in the development of cutaneous SCC. [3-4,10-11,13] Solar elastosis is a non-neoplastic UV-induced lesion

commonly observed in poorly-pigmented skin of horses and humans with or without SCC. It is defined as an accumulation of abnormal elastin in the dermis and is histologically characterized by aggregates of thick interwoven elastic fibers mixed with degenerate collagen within the superficial to mid dermis as observed in our case. The pathogenesis of elastin accumulation is unclear, but is often seen in poorly pigmented or non-pigmented skin with chronic UV exposure, or in conjunction with other solar induced changes such as solar (actinic) keratosis, epidermal plaques, and SCC. In horses, solar-associated SCC has been reported in the conjunctiva, eyelid, and vulvar epithelium, and is morphologically similar to solar-induced SCC in humans, often exhibiting solar elastosis within or adjacent to the neoplasm.[3] Chronic UV-exposure evidenced by solar elastosis has been observed in human cases of clear cell SCC, and is hypothesized to play a role in the development of the disease.[13] The solar elastosis observed in our case parallels that of human clear cell SCC, and suggests that chronic UV exposure may play an etiologic role in the development of clear cell SCC in horses as well.

Contributing Institution:
Michigan State University Veterinary Diagnostic Laboratory and Department of Pathobiology and Diagnostic Investigation, College of Veterinary Medicine, Michigan State University, East Lansing, MI, USA https://dcpah.msu.edu/

JPC Diagnosis: Haired skin, eyelid: Squamous cell carcinoma, clear cell variant.



Eyelid, horse. Neoplastic cells measure up to 50um in diameter with peripheralized, hyperchromatic nuclei. HE, 400X)

JPC Comment: This is a unique neoplasm in the experience of the JPC Vet Path Service. Since the original submission of this case, it has been published as a case report in the Journal of Veterinary Diagnostic Investigation.[17]

This case was reviewed by human subspecialty pathologists at the JPC, who rendered the following consultation – "Interesting case. Based on morphology, we believe a lot of the clear cells are cytologically bland and there are areas of definite tubular differentiation, favoring a malignant clear cell hidradenoma. Additional myoepithelial stains would favor an adnexal origin rather than squamous cell carcinoma. Less likely would be a metastatic clear cell carcinoma such as metastatic renal cell carcinoma[18]. This is not favored based on morphology, but there seems to be co-expression of vimentin and cytokeratin in the section that we reviewed."

Hidradenoma, a term far more commonly used in human rather than veterinary pathology, generally refers to neoplasms of eccrine sweat glands, which the moderator pointed out are not present in the horse (except in the portion of the hoof known as the frog). More recent investigations into the etiology of hidradenomas in humans have postulated potential origins from apocrine sweat glands as well.)

The consensus of the attendees is that while there is a focus of tubular differentiation of neoplastic cells at the deep margin, the predominant form of differentiation is toward a squamous cell morphology, and agree with the contributor's diagnosis in this case. In addition, areas of solar elastosis were noted in the superficial dermis, lending credence to the contributor's suggestion that is represents a neoplasm induced by chronic UV exposure.

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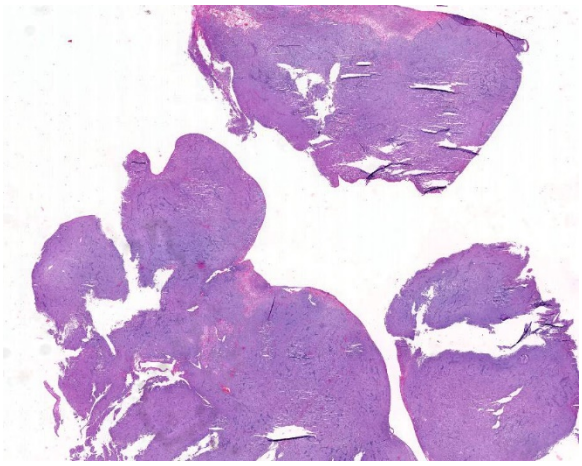
CASE III: Case 2 (JPC 4070611).

Signalment: 2 year 7 month old castrated male Labrador retriever, dog (*Canis lupis familiaris*).

History: This dog presented to the Queen Mother Hospital for Animals (Royal Veterinary College, UK) with a 4 month history of intermittent bloody discharge from his penis. The owner reported no problems with frequency, volume or the action of urination, nor did his urine appear to be discoloured. He had no previous history of illness aside from the presenting complaint. The dog had been imported from continental Europe. Urinalysis, blood biochemistry, and abdominal ultrasound were unremarkable. Haematology revealed a mild increase in lymphocytes, but was otherwise unremarkable.

Gross Pathology: Physical examination revealed a smooth swelling at the base of the penis, which upon examination under general anaesthesia appeared to be a friable mass within the prepuce. The mass was removed and submitted for histopathologic examination.

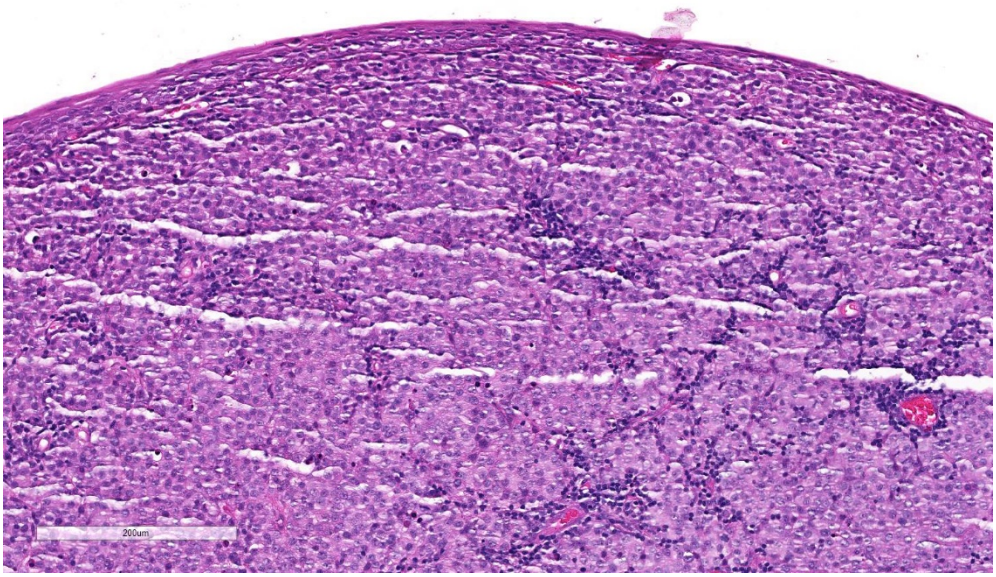
Laboratory results: Cytologic examination of five impression smears reveals high nucleated cellularity, moderate amounts of blood and low numbers of lysed cells on a pale basophilic background. High numbers of round cells are found on all smears. Cells have a round to oval nucleus, 10-15um in diameter with finely to coarsely stippled chromatin, often a single large, round, prominent nucleolus and moderate amounts of pale blue cytoplasm, frequently containing



Prepuce, dog. Multiple sections of an exophytic neoplasm are submitted for examination. (HE, 4X)

moderate numbers of punctate vacuoles. Cells display mild to moderate anisocytosis and anisokaryosis, with low numbers of mitotic figures and rare binucleated forms. Low to moderate numbers of small lymphocytes are focally admixed with the tumour cells. Two smears additionally contain moderate to high numbers of squamous epithelial cells in sheets and individually, often displaying dyskeratosis. Squamous cells display mild anisocytosis and anisokaryosis, occasional bi- and rare multinucleation, and occasionally prominent nucleoli. Also occasional cells have prominent perinuclear vacuolation. These two smears also focally contain high numbers of variably degenerate neutrophils, occasionally containing intracellular cocci. Low numbers of macrophages are also found. These features are consistent with transmissible venereal tumour, with septic neutrophilic inflammation and squamous dysplasia. Other round cell tumours (e.g. of histiocytic origin) cannot be ruled out completely, but far less likely. Septic inflammation and squamous dysplasia are likely secondary changes to the presence of the tumour (e.g. ulceration).

Microscopic Description:
Multiple sections of non-haired skin, from the prepuce per history, are examined. Effacing the lamina propria, raising a multifocally eroded and attenuated stratified squamous epithelium, and extending to the lateral and deep borders of the examined sections, is an unencapsulated, poorly demarcated, round cell neoplasm. The cells are arranged in sheets and loose cords amongst a scant fibrovascular stroma. The neoplastic cells are round with indistinct cell borders, a moderate amount of eosinophilic cytoplasm containing small clear vacuoles, a central round nucleus with stippled or peripheralised chromatin, and a single prominent magenta nucleolus. Anisocytosis and anisokaryosis are mild, and 42 mitoses are observed in 10 high power (x400) fields, some of which are bizarre. Multifocally the tumour is infiltrated by moderate numbers of lymphocytes and plasma cells. Moderate to high numbers of extravasated erythrocytes and fibrin (haemorrhage) are present beneath the epithelium multifocally.

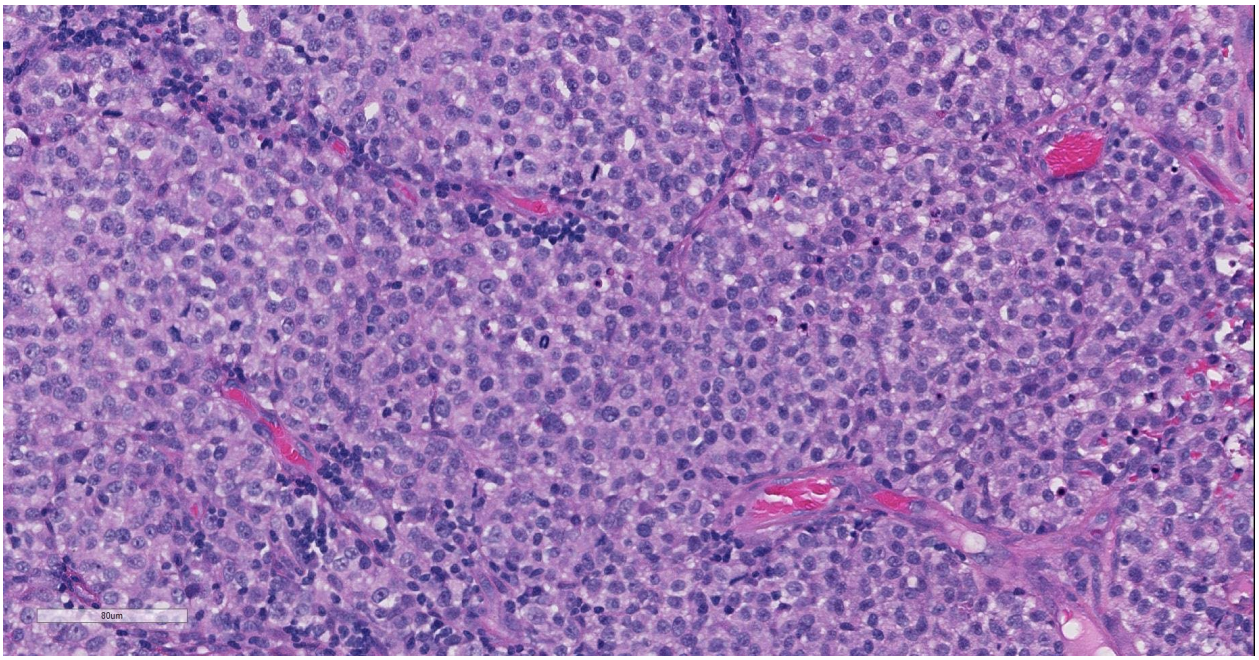


Prepuce, dog. Neoplastic cells abut an intact mucosal membrane. (HE, 40X)

Contributor's Morphologic Diagnoses: Prepuce; Transmissible venereal tumour.

Contributor's Comment: Histopathologic and cytologic examination reveals a canine transmissible venereal tumour (CTVT). The cell of origin in CVTVs is unknown,1 however a histiocytic origin is suspected due to positive immunolabelling for lysozyme, ACM1, a-1-antitrypsin and vimentin, and negative immunolabelling for keratin, desmin a-smooth muscle actin, CD3, lgG and lgM, and A and K light chains.[10,13] CTVT should always be considered in cases of genital round cell tumours and primary differentials are other round cell tumours of the skin, such as histiocytoma, lymphoma and poorly granulated mast cell tumour.[5] Haematoxylin and eosin differentiation between histiocytomas and CTVTs can be challenging, and cytology should be used, as in this case, to help confirm the diagnosis because of better nuclear preservation in cytologic preparations.[5]

CTVT is the most common tumour of the penis of dogs,[7] and typically affects the proximal part of the penis.[3] The tumour typically presents as a single or multinodular mass[4] with a red ulcerated surface.[5] Although primarily located on the genitalia,[5] tumours can also arise on the oral, nasal or conjunctival mucosa.[7] Less commonly the skin is involved,[7]particularly sites which can come into contact with the genitalia.[5] Dogs of both sexes and all ages can be affected, and it is most common in the dorsal vagina[15] of



Base of penis, dog. Neoplastic round cells have abundant vacuolated eosinophilic cytoplasm with large centrally placed round nuclei and prominent nucleoli. Mitotic rate is 3-5 per HPF, and there are infiltrates of lymphocytes within the fibrovascular stroma. (HE, 400X)

sexually mature female dogs.[5] The pathogenesis is by physical transplantation rather than infectious means, and usually occurs during coitus.[5] This is thought to be due to abrasions and bleeding of penile and vaginal mucosa during coitus increasing the chance of transplantation.[13] Similar to canine cutaneous histiocytomas, the tumour has a predictable growth pattern of progressive growth, a static phase, and then regression after one to two months.[10,13] Lymphoplasmacytic infiltration is a feature during regression[7] and can be used to distinguish from lymphoma.[5,13] Metastasis is rare, but has been reported in immunosuppressed animals and puppies. Metastases to the inguinal lymph nodes, brain, adenohypophysis, liver, and kidneys have been reported.[6,13]

The global distribution of CTVT is unexplained, and is previously unreported in the UK.[5] Two subclades of CTVT have been identified, each of which now has a broad geographic distribution.[5]The host does not contribute to the growth of the tumour.[7]

The neoplastic cells have 59 rather than the usual 78 chromosomes present in the cells of dogs, and this karyotypic variation is consistent between CTVTs of different animals.[7] A 1.5kbp repetitive insertion has been identified upstream of c-myc in all CTVTs.[7] Further investigation has revealed that CTVT first arose from a common ancestral neoplastic cell in a wolf or dog related to 'old' East Asian breeds.[13] This is thought to have occurred 200 to 2500 years ago, thus CTVT may represent the oldest known mammalian somatic cell in continuous propagation.[13] CTVT can be transmitted between other canids, including foxes, coyotes, jackals and wolves.[13] Dogs appear to be resistant to challenge after natural regression of the tumour.[15]

CTVT was the first tumour to be transmitted experimentally, by Novinsky in 1863.[15] In 1965, a transmissible sarcoma, both

spontaneous and induced by 20-methylcholanthrene, in Syrian hamsters was successfully transmitted experimentally between individuals without immuno-suppression of the host.[2] Two other transmissible tumours have also been identified. Devil facial tumour disease is a transmissible tumour of the Tasmanian devil (*Sarcophilus harrisi*). lmmunohistochemical profiling has demonstrated a Schwann cell origin of this monophyletic clonally transmissible tumour.[11] A Schwann cell specific myelin protein, periaxin (PRX), is used as a diagnostic marker for devil facial tumours.[11] Finally, a clonal contagious leukaemia in soft shelled clams (*Mya arenaria*) exhibits characteristics similar to CTVT: The genotype of neoplastic cells does not match the host animal, and there is near identical genotype of neoplastic cells between affected animals despite geographic separation, highly suggestive of horizontal transmission of leukaemia in these clams.[8]

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JPC Diagnosis: Prepuce: Transmissible venereal tumor.

JPC Comment: Transmissible venereal tumor occupies a unique niche in cancer biology as the most well-characterized of a small subset of neoplasms which is transmitted as living cells between hosts, rather than transformation of the host's cells.[1] The contributor has provided an excellent review of this tumor and other transmissible tumors in animal species.
The tumor, which has been reported under several names, to include transmissible venereal sarcoma, venereal granuloma, infectious sarcoma, and Sticker's sarcoma, was first described by Hujard in 1920.[4] In 1876, the Russian veterinarian Novinsky demonstrated the nature of the tumor with the first transplantation experiments.[4]

A 2015 paper by Setthawongsin et al[16] detailed molecular identification of the specific long interspersed element (LINE) inserted upstream of the c-myc gene in CTVT cells via PCR on frozen tissue. The moderator discussed recent development of a PCR test for paraffin-embedded tissue utilizing primers of less than 100 bp which is now publicly available through the Michigan State Veterinary Diagnostic Laboratory, which may be of utility in diagnosing neoplasms not present in traditional genital locations.

The moderator also compared cytologic versus histologic appearance of these neoplasms. When taken in context of being from the genital area, the diagnosis of CTVT is not especially difficult. In this particular sample, the cytologic appearance with traditional round centrally placed nuclei, prominent, often single nucleoli, and the presence of lymphocytes and plasma cells often in perivascular location is very characteristic of this particular neoplasm. Cytologically, the presence of numerous vacuoles within the blue cytoplasm of round cells is also a fairly characteristic finding in CTVT, and some pathologists prefer cytology over histology for their diagnosis.

CTVT has evolved a number of mechanisms to evade the host response. Tumor cells downregulate DLA class II expression, but express just enough (10%) of DLA class I genes (complete masking of DLA Class I genes actually activates natural killer cells).[4] TVT cells can also secrete immuno-suppressive levls of transforming growth factor-β. During the period of regression,

host IL-6 liberated from infiltrating lymphocytes works with host IFN-γ to re-establish DLA expression.[4]

Since the submission of this report, other clonally transmitted tumors have been reported in bivalves, including bay mussels (*Mytilus trossulus*), cockles (*Cerastoderma edule*) and golden carpet shell clams (*Polititapes aureus*).[9] Interestingly, the neoplasm in golden carpet shell clams appears to have arisen in another species, the pullet carpet shell (*Venerupis corugata*).[9] Even more interesting is a 2015 report by Muelenbachs et al.[14] of pulmonary and lymph node neoplasms in a 41-year-old HIV-positive individual with concurrent *Hymenolepis nana* infection. Neoplasms composed of small, rapidly dividing cells uncommon in human pathology were determined, after a CDC consultation, the neoplasms were determined to contain DNA of the human cestode *Hymenolepis. nana*.[14]

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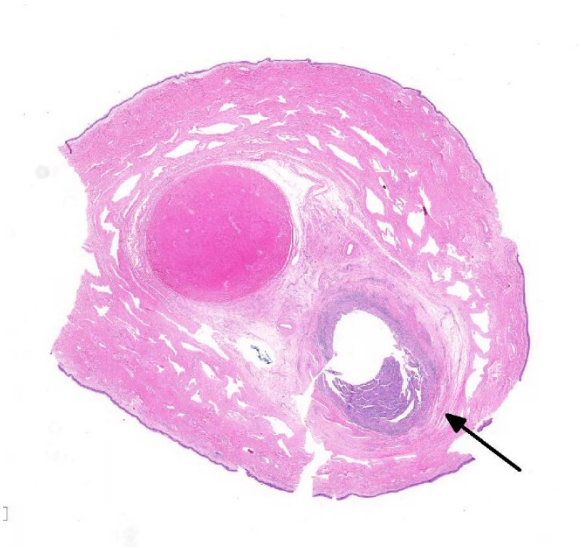
CASE IV: C9969-15 (JPC 4070611).

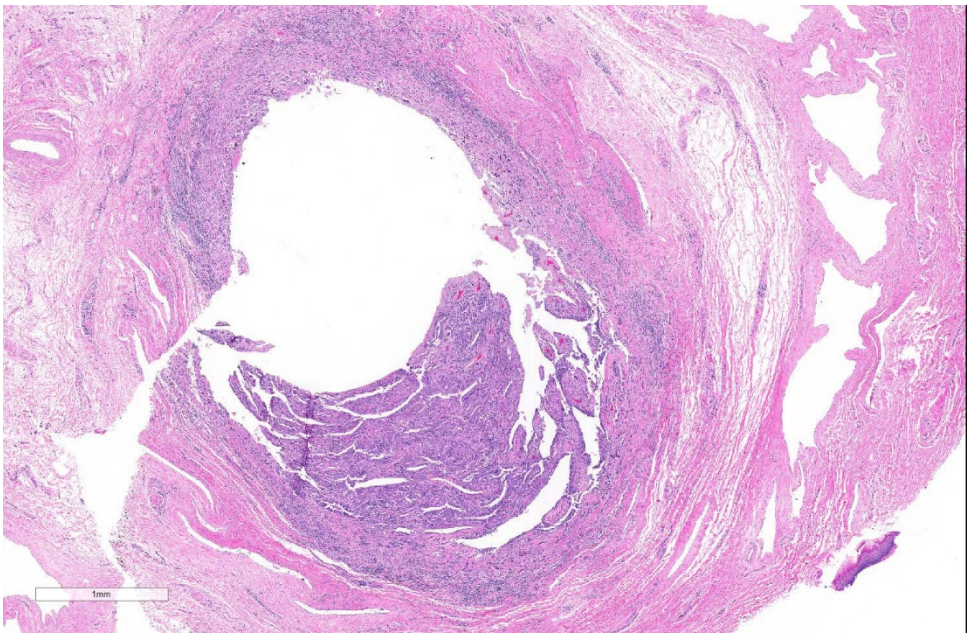
Signalment: 11 year old, male, castrated, Labrador retriever, *Canis lupus familiaris,* canine

History: The dog presented to referring veterinarians with hematuria and dysuria. A cystotomy was performed and calculi were removed. Several months later Rudy presented again with hematuria. He would also dribble small spots of blood from the preputial opening when not urinating. Otherwise, the dog was bright, alert and responsive. The dog was referred to the Atlantic Veterinary College teaching hospital for further work-up. A urethroscopy was performed. Approximately 1 cm from the tip of the penis and extending approximately 2 cm further up the urethra, the mucosal surface was roughened with irregular frond-like structures extending into the narrowed lumen. Small pinch biopsies were taken of the abnormal mucosal surface. A diagnosis of urethral urothelial (or transitional) cell carcinoma was made. A partial penile amputation and an urethrostomy at the level of the bulbourethral gland was performed. The amputated portion of the penis was submitted for histopathology.



Penis, dog: The penile urethra is partially occluded by a neoplasm (arrow) and the submucosa is hypercellular. (HE, 5X)

Gross Pathology: A 7.5 cm long section of the distal penis (including the glans and the distal body of the penis) is submitted for examination. The length of the urethra was opened. The mucosal lining of the distal 3



Penile urethra, dog: At higher magnification, the urethral mucosa is circumferentially ulcerated by an exophytic, and infiltrative neoplasm. (HE, 27X)

cm of the urethra was mildly roughened. Several cross-sections through the entire penis were examined.

Laboratory results Presurgical complete blood count and serum biochemistry; mild abnormalities included:

- ¢ RBC 5.26 x 10^{12} /L (normal range: 5.7 - 8.4 10^{12}/L)
- ¢ Hemoglobin 127 g/L (normal range: 135 - 198 g/L)
- ¢ Hematocrit 0.364 L/L (normal range: 0.40 - 0.56 L/L)
- § ALT 93 U/L (normal range: 13 - 69 U/L)

Mild anemia was attributed to mild, chronic blood loss. Mildly increased ALT may represent mild hepatocellular leakage or possibly normal variation in this individual.

Microscopic Description: Microscopically, the urethral lumen is narrowed and the mucosa is irregularly thickened by an infiltrative, poorly defined epithelial tumor composed of many small nests, occasional small acini and few small papillary projections covered by large polygonal neoplastic epithelial cells. These infiltrates are supported by small amounts of dense fibrous stroma containing mild to moderate infiltrates of plasma cells, neutrophils, fewer lymphocytes and macrophages. The mucosal lining is extensively ulcerated and occasionally covered by small amounts of proteinaceous debris. Several layers of pleomorphic neoplastic epithelial cells partially line the urethral lumen in areas. Small clusters and scattered individual polygonal tumor cells frequently breach the mucosal basement membrane and infiltrate the underlying submucosa where they are accompanied by mild multifocal infiltrates of lymphocytes, plasma cells, few neutrophils, few small foci of hemorrhage and occasional macrophages rarely laden with hemosiderin. Neoplastic epithelial cells have medium to large, ovoid, nuclei with finely stippled chromatin, one to several, prominent nucleoli and moderate to large amounts of poorly defined, cytoplasm. Anisokaryosis is moderate to severe. Mitotic figures are present (7 per 10 HPF). Small clusters of neutrophils admixed with a few sloughed tumor cells are noted in the urethral lumen. Rarely, small clusters of polygonal

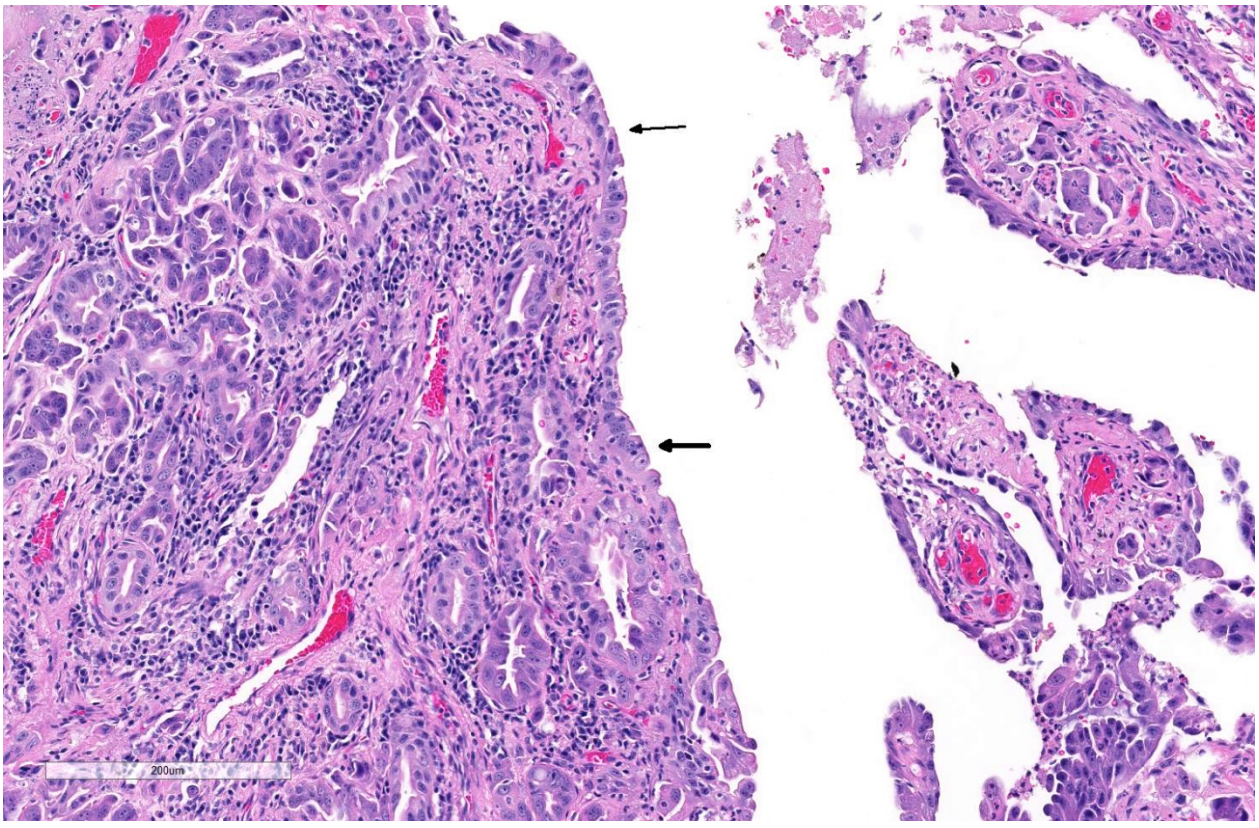
cells resembling tumor cells are present within lymphatics and veins within the submucosa, the corpus spongiosum and the pars longo glandis (not present in all sections). The ovoid, discrete aggregate of dense, poorly cellular, collagen dorsal to the urethra represents the distal end of the os penis.

Contributor's Morphologic Diagnoses: Penis: 1. Urethral urothelial (or transitional) cell carcinoma

2. Mild to moderate, lymphoplasmacytic and neutrophilic, erosive, chronic, urethritis

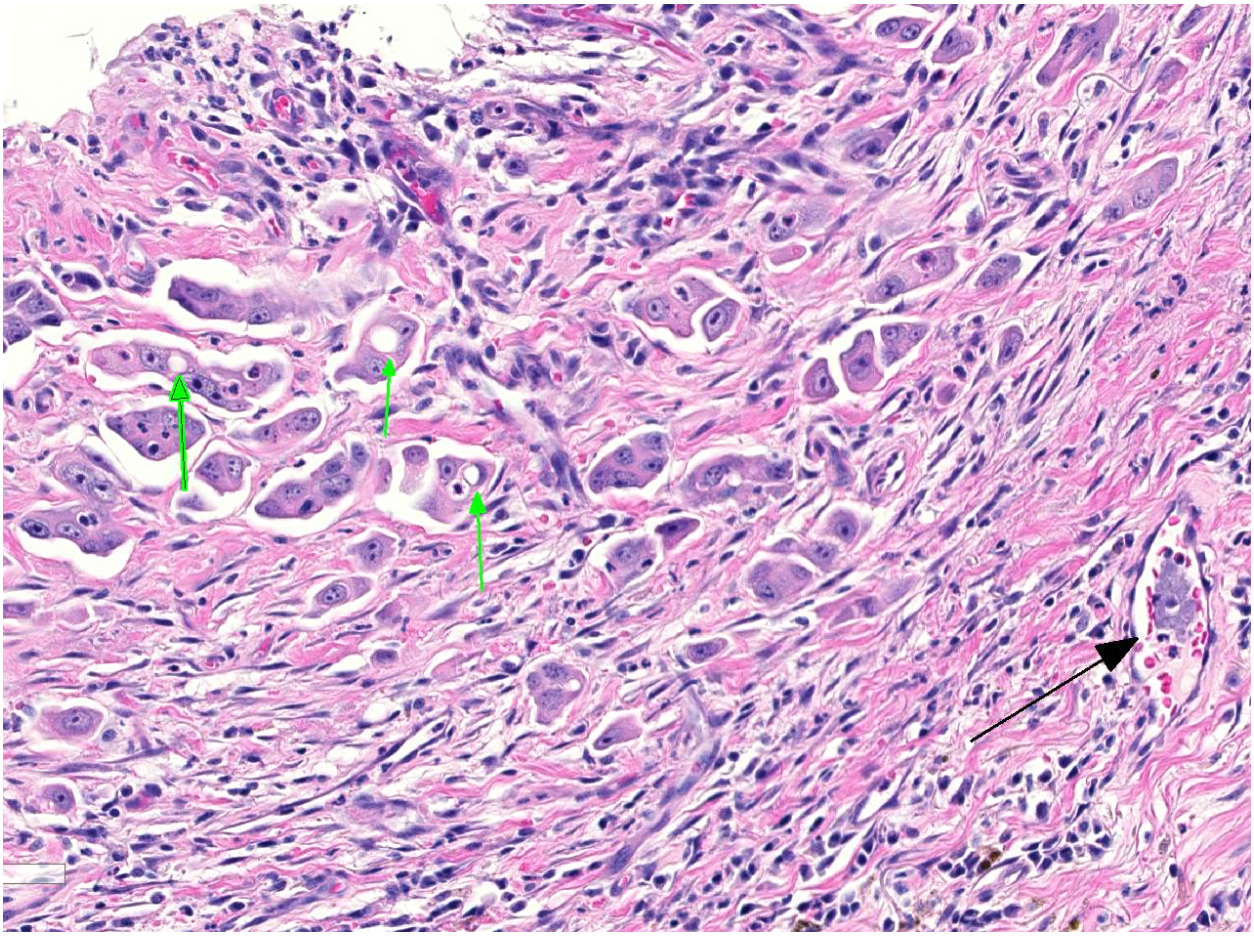
Contributor's Comment: The cause of hematuria and persistent blood dribbling from the penis of this dog was a urothelial (or transitional) cell carcinoma arising within the distal urethra. Ultrasound performed at the time of surgery revealed no abnormalities in the urinary bladder and no evidence of abdominal or retroperitoneal lymph node enlargement. Thoracic radiographs were also unremarkable. Occasional small microscopic clusters of putative tumor cells were noted within vascular structures in these sections. Despite the latter finding, 2 years after partial penile amputation and complete removal of the tumor, this dog is still alive and has exhibited no clinical signs indicative of metastatic disease (per recent discussion with the rDVM).

Primary urethral tumors are very rare in dogs. The vast majority of these tumors are



Penile urethra, dog: Neoplastic epithelial cells form nests, glands, and line papillary fronds in the exophytic portion of the neoplasm. The dense stroma is infiltrated by moderate numbers of lymphocytes and plasma cells with fewer neutrophils. (HE, 220X)

malignant and most are urothelial cell carcinomas. Females are generally thought more commonly affected than males. The cause is unknown but a variety of chemical carcinogens have been associated with the development of lower urinary tract tumors in dogs.[2] An association between chronic urethritis and urothelial tumors in females has also been shown.[1] The latter is interesting as this male dog, also had a chronic history of urinary struvite calculi. Urothelial cell carcinomas in the lower urinary tract generally occur in older dogs which often present with hematuria and dysuria. References generally report that metastasis is seen in approximately 30% of cases; regional lymph nodes are the most common site. Approximately 1/3 of dogs with urethral tumors also have urothelial cell carcinomas in the urinary bladder. When present in both sites, affected dogs tend to have a much poorer prognosis and significantly shorter survival times.[1,2] One retrospective study on canine lower urinary tract tumors reported that the longest survival times (median of 365 days) were achieved in dogs with no evidence of metastasis at the time of surgery, that had a solitary carcinoma located in either the urinary bladder or the urethra, and in which complete resection was achieved.[2]



Penile urethra, dog. In ulcerated areas of the mucosa, neoplastic cells infiltrate the inflamed submucosa. Occasional neoplastic cells contain cytoplasmic Melamed-Wolinska bodies (green arrows). Neoplastic cells are present within submucosal vessels (black arrow). (HE, 220X)

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JPC Diagnosis: Penile urethra: Transitional cell carcinoma

JPC Comment: Transitional cell (or urothelial carcinoma)as account for approximately 2% of cancer in the dog. It is the most common neoplasm of the bladder, and the majority are high-grade neoplasms. It is primarily seen in older dogs between the ages of 9 and 11, and most tumors are considered high-grade neoplasms. Scottish terriers have an increased breed predisposition being 18-20X more likely to develop this tumor than other breeds. The trigone is the site of most tumor development, although the prostatic urethra and lower areas of the urethra (as seen in this case) are also commonly affected. Grossly, most neoplasms in the bladder are solitary, although they may cover a large region of the bladder making resection impossible. [1]

Histologically, most tumors are readily diagnosed histologically, especially if an adequate sample is obtained. One of the more diagnostic features is the presence of Melamed-Wolinska bodies, which impart a signet ring appearance to neoplastic cells and are commonly seen in these tumors. These bodies often stain positively for uroplakin (a commonly used immunohistochemical marker for TCC).[1]

Tumor growth patterns often impact on grading schemes. While most neoplasms are infiltrative indicating a high-grade, non-infiltrative neoplasms (carcinoma *in situ*) is occasionally seen or seen at the periphery of infiltrative lesions. Tumors may also be papillary or non-papillary, although this particular feature has not been identified as predictive of future behavior. [1]

Invasive TCC is also extremely prone to widespread metastasis with studies demonstrating a 50-90% rate, most often to the lungs and lymph nodes. Approximately 75% are staged at T2 under the TNM system , and an addition 20% at stage 2 with invasion of adjacent organs or tissues. Cutaneous metastasis has been reported to occur in up to 10% of cases. While the presence of urethral involvement does not appear to be significantly related to the presence or absence of metastatic disease, concurrent presence of TCC within the bladder and urethra is associated with the shortest survival times.[1]
As the prostatic urethra is a common site of urothelial carcinoma and local invasion, the diagnosis of prostatic carcinoma should likely include immunohistochemistry for uroplakin to rule out other forms of carcinoma. Uroplakin (uroplakin III) is an excellent marker for urothelium but may be positive in normal, hyperplastic and neoplastic urothelium. In the dog, staining is seen primarily at the surface of cell membranes in the dog and is often not uniform throughout neoplasms.[1] Other immunohistochemical markers that have been shown to have utility in the diagnosis and prognosis for human and canine TCCs include cytokeratin 7 and cyclooxygenase-2. It should be noted that there is often a loss of uroplakin and cytokeratin expression in high-grade carcinomas, especially infiltrative tumors. [3]

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