Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2017-2018

C o n f e r e n c e 14

10 January 2017

CASE I: BB364/11 (JPC 4019414).

Signalment: 9-year-old, female, spayed, Collie cross, *Canis familiaris*, canine.

History: This dog was referred to the Royal (Dick) School of Veterinary Studies, University of Edinburgh, with a two-month history of persistent watery diarrhea, weight loss and vomiting. The dog had been imported from Italy under the UK's Pet Passport Scheme in 2001. On initial presentation, the dog was bright and alert but thin. The only other physical abnormality was palpable thickening of a loop of bowel in the cranial abdomen. On routine hematology there was lymphopenia and a single biochemical abnormality of a moderately increased ALT (310 IU/l, ref 21–102 IU/L). Abdominal ultrasound confirmed thickening of the jejunal wall. Exploratory celiotomy confirmed marked thickening of one third of the jejunum but found no evidence of obstruction. Biopsies were taken on two separate occasions. The first set consisted of small, full-thickness biopsies from which the initial diagnosis was made; the second set consisted of three portions originating from an 80cm resected segment of thickened jejunum. The dog

Intestine, dog. In the affected segment, the mucosa is circumferentially thickened by firm, pale yellow tissue thickening the intestinal wall up to 3.5cm. (Photo courtesy of: Veterinary Pathology Unit, Division of Veterinary Clinical Sciences, Roslin Institute and Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Easter Bush Veterinary Centre, Roslin, Midlothian, EH25 9RG, UK http://www.ed.ac.uk/schools-departments/vet/services/pathology)

recovered well and has remained asymptomatic.

Gross Pathology: Gross lesions were most appreciable in the portions of resected jejunum taken at the second surgery. In the most severely affected segment, the wall was thickened up to 3.5cm. On transverse

section, this thickening was circumferential and consisted of firm, pale yellow tissue.

Laboratory results:
Immunohistochemistry with antibody against NSE confirmed the abnormal presence of neural elements in the small intestinal lamina propria, most notably clusters of neuronal cell bodies but also streaming bundles of axons.

Microscopic Description: Small intestine (jejunum): Numerous well differentiated neuronal cell bodies are scattered throughout the intercryptal lamina propria. The cell bodies are polygonal with faintly visible cell borders, a moderate amount of cytoplasm, large, oval, eccentrically located, hypochromatic, vesiculated to open-faced nuclei and clearly visible nucleoli. There are numerous short streams and nodular aggregates of elongated cells with abundant eosinophilic, fibrillar to foamy cytoplasm, compatible with bundles of axons or Schwann cells. They form nests within the subcryptal area but extend as streams into the mid lamina propria. The myenteric and submucosal plexuses are increased in number and size. There is moderate goblet cell hyperplasia and the lumen contains abundant fibrillar, eosinophilic, mucinous

*Intestine, dog. Subgross of the affected segment. (HE, 5X)*

material (mucus). In some sections, rare dilated crypts contain small amounts of necrotic cell debris.

Note: Sections from the most severely thickened portion were a little different (not included here). In addition to the above lesions, there was marked superimposed mucosal hyperplasia, characterized by an increased number of well differentiated but elongated or branching crypts, often lined by numerous goblet cells. Some crypts were distended by necrotic cell debris and/or mucus. Crypt rupture had led to release of lakes of mucus and necrotic cell debris into the lamina propria. There was also marked villous blunting and fusion, with ulceration.

Contributor's Morphologic Diagnosis:
Jejunum: Intestinal ganglioneuromatosis

Contributor's Comment: Intestinal ganglioneuromatosis (GN) is a rare condition in which there is hyperplasia of all components of the intestinal ganglia. It has some similarities with ganglioneuroma, which is also the result of neuronal and axonal proliferation but is considered neoplastic.[7] However, in contrast to ganglioneuroma, which tends to be well defined and mass-like, GN is poorly demarcated and more diffuse.[5] In humans, diffuse GN presents in one of two forms, transmural or mucosal. Transmural lesions also have a more guarded prognosis due to their link with multiple endocrine neoplasia (MEN) IIb and neurofibromatosis 1 (NF1).[5,19] GN usually arises in the colon or rectum in humans, though one report described lesions extending from lips to rectum.[3,19] Reported clinical signs have included vomiting, weight loss, diarrhea or constipation, hematochezia, melena and abdominal pain.[1,4] Similar clinical presentations have been described in veterinary patients.[7,8]
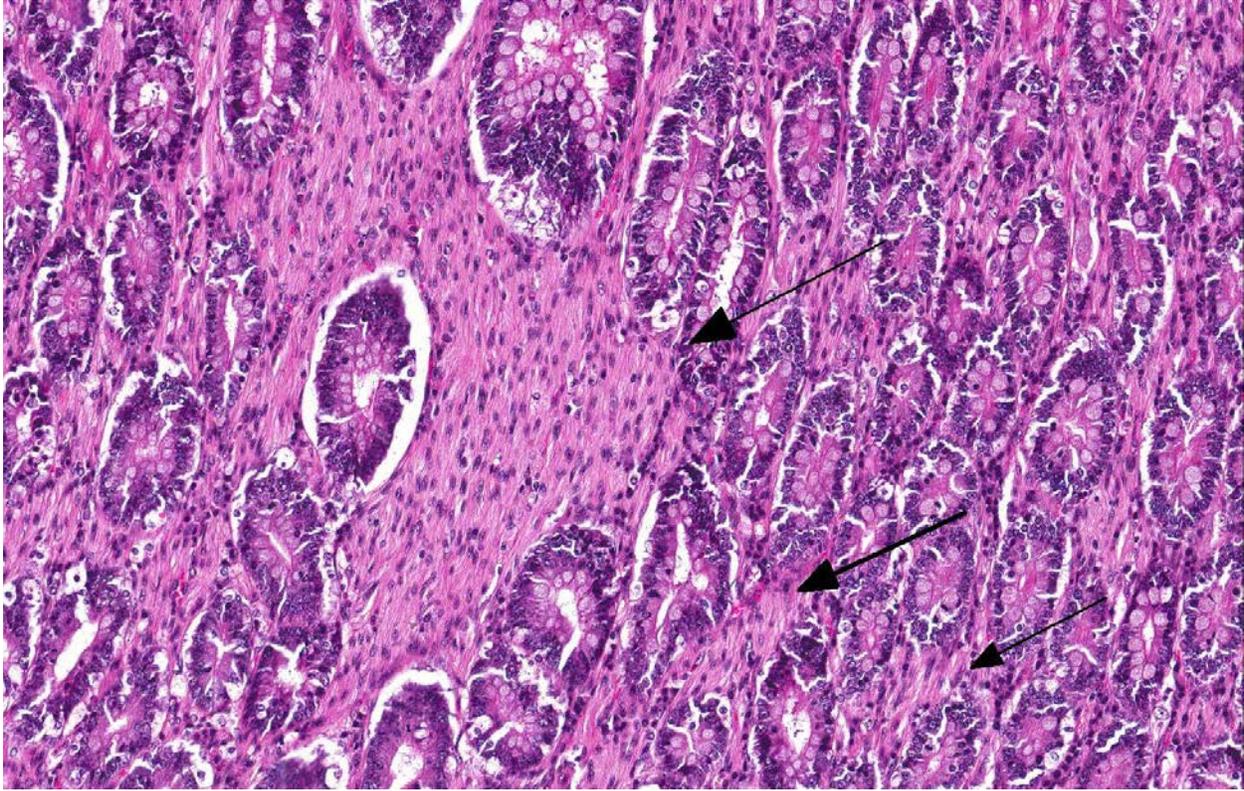
Intestine, dog. Intestinal crypts are separated and occasionally replaced by nerve bundles (arrows) with accompanying Schwann cells. (HE, 200X)

The few previous reports of canine GN have been in juveniles and have typically only involved the colon.[2,7] Small intestinal GN is very rare in the dog, with only one report in the literature.[8] In general, alimentary GN in the dog tends to mirror the transmural form in humans, involving the submucosa and intestinal wall, with relative sparing of the mucosa. Our case was a little different in that the lesion arose in a mature dog with no prior history of gastrointestinal disease; it predominantly affected the mucosal lamina propria of the small intestine; and it was successfully treated. In all prior cases it has been speculated that the lesions are probably congenital, based upon the age of the patient and the slowly progressive nature of the disease.[2]

As mentioned above, MEN IIb, which is the result of a point mutation in the RET proto-oncogene, accounts for the majority of intestinal GN cases in humans. However, the exact pathogenesis of GN is still unclear. Proposed theories include overexpression of neural growth factor leading to proliferation of one nerve fiber type; hyperplasia of multiple nerve fibers, rather than "clonal expansion" of a single subtype; decreased expression of the tumor suppressor gene, PTEN; and increased expression of glial cell line-derived neurotrophic factor (GDNF) and a related neurotrophic factor, neurturin.[6,9,12,16] In humans, clinical signs attributable to GN develop within the first weeks of life and are followed by skeletal abnormalities, thickened lips, mucosal neuromas, and a Marfanoid habitus. Multiple endocrine neoplasms occur in later life, including medullary thyroid carcinoma and pheochromocytoma.[10] There were no clinical signs to suggest multiple endocrine neoplasia in this case and the adrenal glands were ultrasonographically normal. The
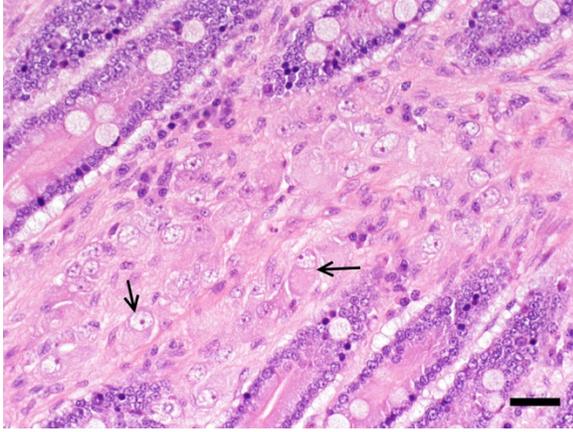
Intestine, dog. Well-differentiated neurons are scattered through the neuronal tissue within the lamina propria (arrows). (HE, 400X) (Photo courtesy of: Veterinary Pathology Unit, Division of Veterinary Clinical Sciences, Roslin Institute and Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Easter Bush Veterinary Centre, Roslin, Midlothian, EH25 9RG, UK http://www.ed.ac.uk/schools-departments/vet/services/pathology)

etiology of diarrhea associated with neural tumors is still unclear. Increased levels of vasoactive intestinal polypeptide (VIP) have been identified in some cases of human GN, resulting in the clinical syndrome of watery diarrhea, hypokalemia and achlorhydria.[11] Alternative mechanisms for the diarrhea include altered intestinal motility, hypersecretion and malabsorption.

JPC Diagnosis: Jejunum: Ganglio-neuromatosis.

Conference Comment: Intestinal ganglio-neuromas arise in peripheral ganglia and are composed of well-differentiated neurons and nerve processes, Schwann cells, and enteric glial cells. Exceedingly rare in domestic animals, intestinal ganglioneuromatosis (GN) is characterized as regional or segmental proliferation of ganglio-neuromatous tissue and has only been reported in dogs, a Boer goat[18], a piglet[17], and a horse[15]. Immuno-histochemical stains aid in the identification of the individual cell types, with neurons positive for neuron

specific enolase (NSE) and S-100 and Schwann and enteric glial cells positive for S-100 and glial fibrillary acidic protein (GFAP).[15] These benign proliferative lesions occur predominately in the ileum or colon with few reports of small intestinal infiltration[13] and can usually be associated microscopically with the myenteric plexus from which they either extend through the outer portion of the tunica muscularis to the serosal surface[15] or through the inner portion of the tunica muscularis, submucosa, and muscularis mucosa, and into the lamina propria[13] where it can result in chronic-intestinal pseudoobstruction (CIPO) due to polypoid to segmental expansion of the lamina propria.[14] The pathogenesis of GN is currently unknown; however, researchers have identified deletion of PTEN in the enteric nervous system (ENS) of affected mice which corresponds to abnormally low PTEN expression in humans with enteric GN causing CIPO. PTEN is a phosphatase that controls cell growth, proliferation, and death. In these mice, the clinical changes of CIPO were reversed by administration of a
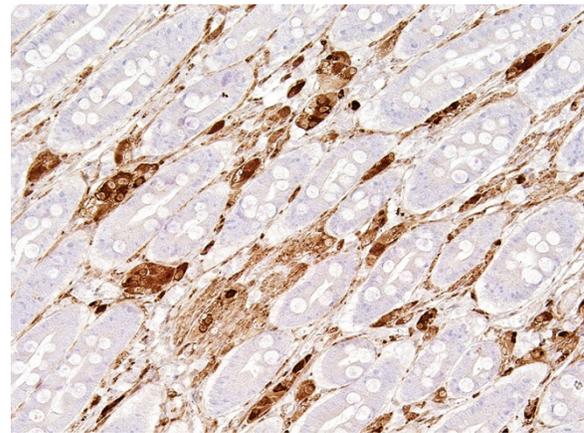
Intestine, dog. Neurons stain immunopositively for neuron-specific enolase. (Photo courtesy of: Veterinary Pathology Unit, Division of Veterinary Clinical Sciences, Roslin Institute and Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Easter Bush Veterinary Centre, Roslin, Midlothian, EH25 9RG, UK http://www.ed.ac.uk/schools-departments/vet/services/pathology)

pharmacological inhibitor of the PI3K/PTEN-AKT-S6K signaling pathway, indicating a potential therapeutic target for ganglioneuromatous forms of CIPO.[14] Additionally, current literature reveals that segmental GN can be removed surgically resulting in resolution of clinical signs and a successful outcome.[13]

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CASE II: 2 (salmon colored slide) (JPC 4101493).

Signalment: 6-year-old, female, Epagneul Breton (French Brittany), *Canis familiaris*, canine.

History: The dog was found dead at home 23 days after parturition. The owner reported that delivery was normal and the dog gave birth to three puppies.

Gross Pathology: At the necropsy, the dog was emaciated and moderate hemorrhagic enteritis and diffuse pulmonary edema were observed. The endometrium was hemorrhagic and thickened by the presence of brown ellipsoidal enlargements (previous placental attachment) distributed in the left and right uterine horns. The uterine lumen contained small amounts of serosanguinous fluid. The cause of death was attributed to development of acute pneumonia.

Laboratory results:
None provided.

Microscopic Description: Uterus, placental sites: The uterine mucosa is expanded by irregular, multilobulated, eosinophilic projections that compress the adjacent endometrium.

The mass is composed by large amount of fibrillary pale eosinophilic dense material (collagen), finely beaded meshwork of fibrillary, pale eosinophilic material (fibrin), moderate amount of extravasated erythrocytes (hemorrhage), and necrotic and karyorrhectic debris accumulating prevalently in the proximal 1/3rd the projections. Between the deepest portion of the eosinophilc matrix and the glandular zone, there are variable numbers of polygonal multinucleated giant cells with an epithelioid appearance characterized by abundant eosinophilic cytoplasm, often vacuolated (decidual cells/syncytial trophoblast). These cells were often oriented
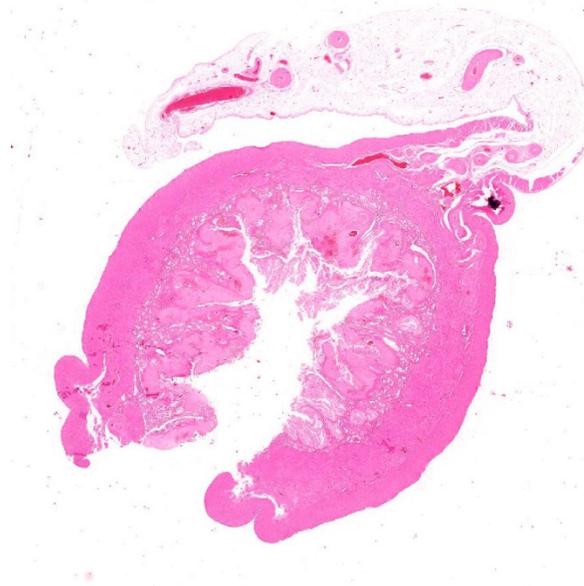
Uterus, dog. A cross section of uterine horn with a markedly thickened endometrium is presented for examination. (HE, 4X)

around vascular structures that were characterized by hyaline walls (vascular degeneration). The glandular zone is characterized by reduced number of endometrial glands, that are multifocally variably dilated and filled with moderate amount of eosinophilic fluid and necrotic and karyorrhectic debris. Within the endometrial mucosa, lamina propria is fibrotic and moderate numbers of mature lymphocytes, plasma cells, and lesser numbers of hemosiderin laden macrophages were also present (mild chronic endometritis). Multifocally, the superficial mucosa lining is sloughed, but when present is organized in papillary projections lined by swollen columnar epithelial cells with abundant, clear, foamy vacuolated cytoplasm and apically located vesicular nuclei (progestational epithelium).

Additional findings (not in the slides): numerous follicles and large corpora lutea were present both ovaries.

Contributor's Morphologic Diagnosis:
Uterus, placental site: Coagulative necrosis, subacute, locally extensive, moderate, with retention of trophoblasts/decidual cells, hemorrhages and mild chronic lympho-plasmacytic endometritis (involution of placental sites).

Contributor's Comment: In dogs, normal involution of the genital tract, after whelping is a slower process compared to other species. More rapid initial involution occurs during the first 4-6 week post-partum. During this period odorless green or dark brown vaginal discharge called "lochia" can be observed (as it was for this dog).

Histologically, placental site involution
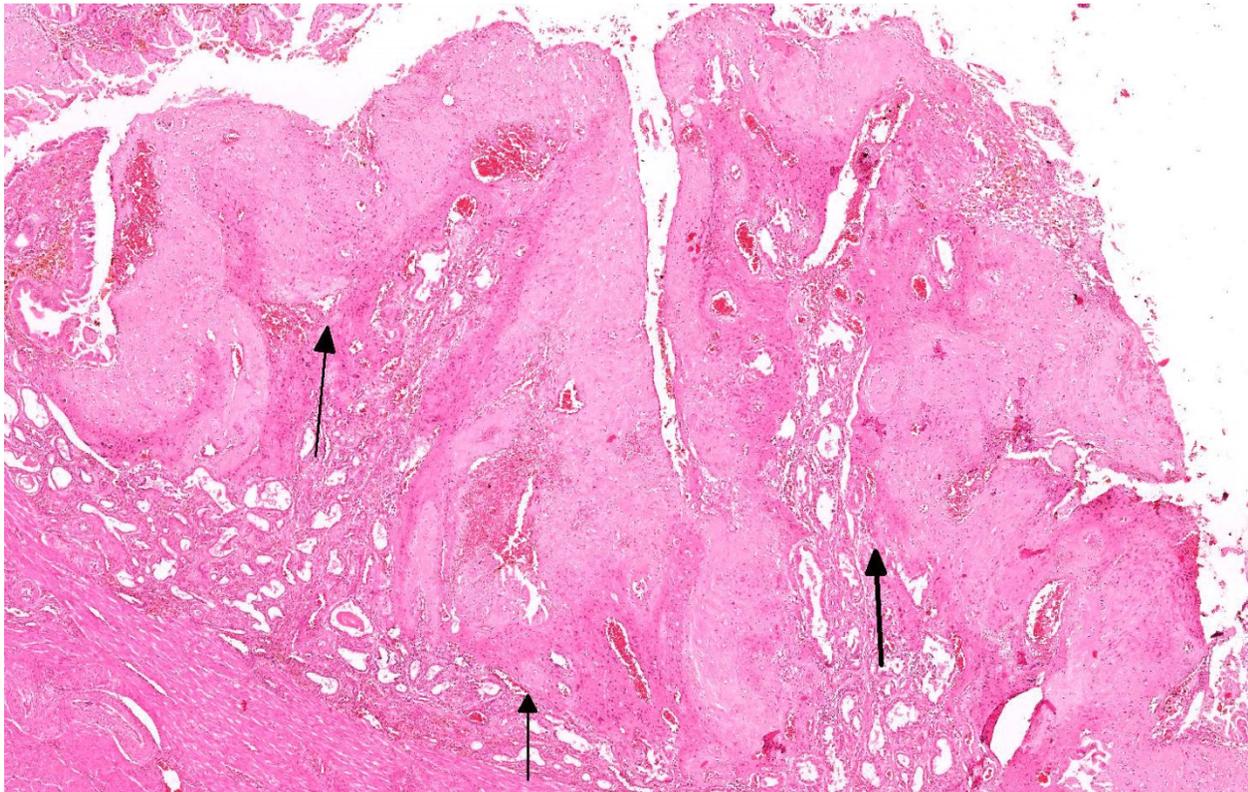
Uterus, dog. Approximately half of the endometrium is replaced by large, coalescing plaques of collagen, remnant trophoblasts, and cellular debris. (HE, 30X)

starts with massive epithelial sloughing into the uterine lumen. Sloughing is at the level of attachment to the endometrial lamina propria that is also expanded by the presence of inflammatory cells (lymphocytes, plasma cells and macrophages).[2] Trophoblasts, on the surface of the uterine mucosa and scattered throughout the lamina propria, are numerous, often necrotic and degenerated, and according to Orfanou et al.,[7] can be observed even at 84 days post-partum. The area of detachment is soon regenerated and covered by a single layer of columnar epithelial cells. During involution, the majority of the uterine glands return to normal size and shape. By the commencement of the eighth week almost all of the collagen masses have sloughed into the lumen. This process continues until the end of the twelfth week and finally, the uterus can be classified as anestrus from the thirteenth week postpartum.[2,7]

Subinvolution of placental sites (SIPS) is a disorder of young, primiparous bitches that causes a sanguineous vulvar discharge any time after the fourth week postpartum. In bitches with SIPS, there is a failure or delay in normal uterine involution or a delay of fetal trophoblasts to regress physiologically.[1,8]

Usually, young bitches are affected, even if the exact etiology of the condition is unknown but factors thought to play a role include failure of thrombus formation and occlusion of endometrial blood vessels due the continuous invasion of trophoblast-like cells into the endometrium and myometrium and the decreased influence of decidual cells on them.[1] SIPS has also been reported in women, and although placentation is hemochorial in humans (versus endotheliochorial in dogs), the pathogenesis of SIPS seems similar. In humans, it has been suggested that important factors in the

pathogenesis of SIPS include poor interaction between extravillous cytotrophoblasts and maternal decidual tissue, the absence of immunoglobulins and complement proteins in subinvoluted vessels, and persistent expression of the anti-apoptosis protein Bcl-2, which prevents apoptosis and thereby promotes maintenance of utero-placental vessels.[3,12]

A consistent histological feature is retention and invasion of trophoblast like cells into the underlying stroma and even into the myometrium,[1] together with the presence of abundant collagen mass, largely necrotic and hemorrhagic, that can extended down to involve the whole mucosa or even part of the myometrium. Moreover, the retained trophoblastic cells do not regress or degenerate, but continue to invade the deep glandular layer or even the myometrium, preventing normal thrombus formation in endometrial blood vessels[6] and can be the reason for the prolonged duration of vulvar discharge observed clinically.

The timing of persistence of trophoblast-like cells in physiological involution and subinvolution of placental sites is debated. According to Al-Bassam and co-workers[1] these cells would be present during the first 2 weeks postpartum but in the case of SIPS they persist for a longer period of time. A recent study however, shows that these cells persist on the surface of the uterine epithelium, the placental tissues, as well as in smears of vulvar discharge during the whole period of involution even up to 84 days postpartum in normal involution of the uterus in the bitch.[7]

Clinically, a chronic post-parturient serous or sanguineous discharge (4 to 16 weeks after parturition or even until the beginning of the next estrous) without any systemic illness is the most common presentation.[6]

Affected animals can become anemic, and the uterus is prone to ascending infections.

Spontaneous regression of normal placental sites usually occurs in bitches in good health without significant anemia.[9] However, dogs should be closely monitored weekly or biweekly with clinical, hematologic and ultrasonographic examinations because of the risk of uterine perforation and peritonitis, albeit rare.[6]

In cases of severe bleeding, blood transfusions and ovariohysterectomy should be immediately considered. Usually the affected bitches are not predisposed to recurrence of the disorder as this is a condition that only affects primiparous bitches.[11]

JPC Diagnosis: Uterus, placental site: Normal placental site involution, Epagneul Breton (French Brittany), canine.

Conference Comment: This case accentuates the importance of a good clinical history. Subinvolution of placental sites (SIPS) looks histologically identical to normal involution.

SIPS occurs in young, primiparous bitches and is characterized clinically by prolonged blood-tinged vaginal discharge due to failure of trophoblasts to regress postpartum resulting in delayed re-epithelialization of the endometrium. Sources vary as to duration postpartum that normal uterine bleeding ceases, some say past 7-10 days[10] and others 1-6 weeks[5], and placental sites should be involuted by the 12th week. Nevertheless, the clinical and microscopic manifestations are diagnostic for this syndrome which currently has an unknown cause.[5] Grossly, the uterine horns (cornua) contain segmental, ellipsoidal, irregular, grey to brown thickenings in areas where the
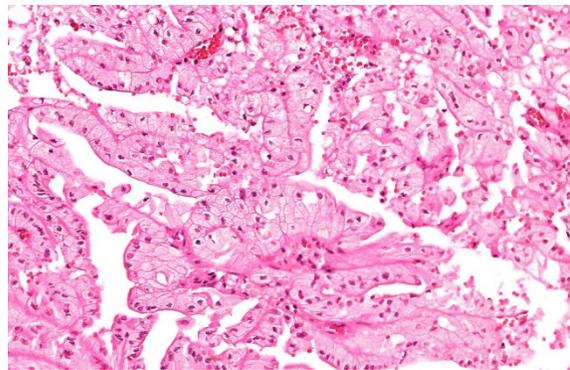
Uterus, dog. Remaining glands are often lined by hypertrophic epithelium with abundant vacuolated cytoplasm (progestational influence). (HE, 226X)

placenta previously attached. The adjacent endometrium is normal grossly and microscopically. Microscopically, these thickenings are composed of a combination of amorphous eosinophilic matrix, fibrin, degenerating placental tissue, and regenerating endometrium.[5] Trophoblasts are more numerous than in normal involuting placental sites and are often aggregated at the base of the fibrous masses and extend into the myometrium even penetrating the serosa and allowing leakage of uterine contents into the peritoneum. The overlying surface epithelium often has a heavily vacuolated cytoplasm indicating the influence of progesterone. Corpora lutea are consistently present in the ovary but progesterone levels are low.[10] Sequella to SIPS are ascending infections, open pyometras, and endometritis. Additionally, in dogs with pre-existing bleeding disorders, like von Willebrand's disease, rapid exsanguination is a gruesome reality.[5]

The composition of the large pink masses largely replacing endometrial tissue within the involuting uterus was a subject of spirited discussion, with some favoring a necrotic coagulum and others favoring a collagenous plaque. In truth, the correct answer is a combination of both. During involution, at approximately 2-3 weeks large

plaques of collagen are formed in the involuting endometrium; these plaques are generally sloughed around 8 weeks.[1] this is consistent with the clinical history in this case of a uterus at 23 days post-partum.

Hamsters and other rodents (see below) have a labryrinthine hemochorial placenta in which the trophoblast cells are in direct contact with the maternal vasculature. Therefore, they routinely have giant trophoblastic cells in the myometrial arteries (they have tropism for arterial blood) and rarely in the pulmonary arteries during gestation and up to 3 weeks postpartum.[4]

Table 1: Placental types by species[4,5,10]

Species	Distribution of contact	Classification by maternal cell layers	Maternal-fetal interdigitation
Mare, Sow	Diffuse	Epitheliochorial	Villi (horse: "cups", pig: folded villi)
Ruminant	Cotyledonary	(Syn)epitheliochorial (combination)	Villi
Bitch, Queen	Zonary	Endotheliochorial	Labyrinth
Rhesus macaque	Double discoid	Hemochorial	Villi
Ape, human	Discoid	Hemochorial	Villi
Rabbits, rodents	Discoid	Hemochorial	Labyrinth

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CASE III: N-1238 (JPC 4102671).

Signalment: 3-month-old, female and male, Boer, *Capra hircus*, caprine.

History: Progressive flaccid paralysis was observed in the hind limbs of 30 out of 40 Boer goat kids over a period of seven days. Age of onset of clinical signs ranged from five to twelve weeks of age; there was no neonatal ataxia. On examination, the kids were affected to varying degrees of severity from hind limb weakness through to an inability to stand. Four kids were found in sternal recumbency. All had normal mentation with intact reflexes and motor function. The mildly affected kids were still suckling. Six animals were euthanized. The herd had no mineral supplementation.

The farm also kept a flock of 120 mixed breed sheep; these had no mineral supplementation and showed no signs of ataxia.

Gross Pathology: Four goat kids were submitted for post-mortem examination (two males [goats A and B] and two females [goats C and D]). They were in relatively good body condition. The lungs were mildly and multifocally mottled red and there was a moderate amount of froth in the trachea (pulmonary congestion and edema). There was a moderate amount of vegetative material within the rumen and well-formed feces in the rectum. Brain and spinal cord were grossly unremarkable.

Laboratory results:
Liver mineral analysis in the four goat kids:
[Reference ranges used from ovine liver (µmol/kg DM) assuming DM 280g/kg:
Mn 130 – 286 µmol/kg DM; Fe 1919 – 19186 µmol/kg DM; Cu 1405 - 5619 µmol/kg DM; Zinc 1639 - 4096 µmol/kg DM; Se 11.3 - 67.8 µmol/kg DM; Molybdenum 11 - 45 µmol/kg DM; Cd 1 – 44 µmol/kg DM; Pb 1- 14 µmol/kg DM; Co 2 -5 µmol/kg DM]

	Mn	Fe	Cu	Zn	Se	Mo	Cd	Pb	Co
	µmol/kg DM	µmol/kg DM	µmol/kg DM	µmol/kg DM	µmol/kg DM	µmol/kg DM	µmol/kg DM	µmol/kg DM	µmol/kg DM
Goat A	177	32182	57 L*	3872	3.6 L	33.7	0.1 L	6.7	3.9
	166	31061	53 L	3734	3.2 L	33.6	0.1 L	6.7	6.6 H*
Goat B	227	18608	57 L	2658	4.4 L	22.7	0.1 L	1.3	2.7
	238	19274	69 L	2785	5.0 L	23.6	0.1 L	1.2	2.9
Goat C	124 L	19243	43 L	2073	4.3 L	26.5	0.1 L	2.4	3.5
	140	23758	125 L	2377	4.3 L	31.5	0.1 L	3.1	5.0
Goat D	159	22351	31 L	3276	3.9 L	26.7	0.1 L	2.3	1.4 L
	189	29184	28 L	4045	3.7 L	33.8	0.1 L	3.3	1.5 L

*L: Low; H: High.

Microscopic Description: The severity of the lesions varies slightly depending on the slide. The cervical spinal cord has bilateral, symmetrical and severe degenerative lesion that affects both grey and white matter. Within the ventral funiculi (mainly the ventromedial tracts) and, less severely, the lateral funiculi, myelin sheaths are markedly distended and often contain axonal fragments and/or few macrophages within its lumen (digestion chambers, Wallerian degeneration). Rare spheroids are also observed. Moderate numbers of neurons located within both the ventral horns and the intermediate area, are swollen and rounded and show loss of Nissl granules which are only remaining at the periphery, with its nucleus displaced towards the periphery (central chromatolysis). Some neurons show karyorrhexis, karyolysis and hypereosinophilia (necrosis). Few small haemorrhages are seen within the grey matter. No inflammatory reaction is observed.

Spinal cord, goat   A section of spinal cord is presented for examination. There is bilateral spongiosis of the dorsal aspects of the ventral funiculi. (HE, 6X)

Similar lesions were observed in sections from cervical, thoracic and lumbar spinal cord from all the goat kids as well as within the brainstem from 2 of them (A and D).
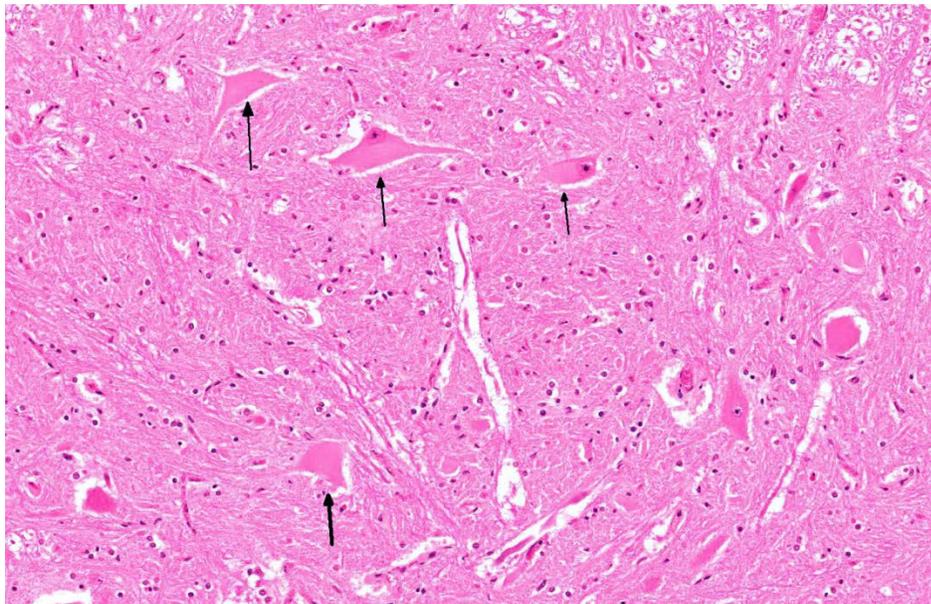
Contributor's Morphologic Diagnosis: Spinal cord (cervical): Severe, multifocal Wallerian degeneration with moderate, multifocal neuronal chromatolysis.

Contributor's Comment: A diagnosis of enzootic ataxia was made based on the histological findings and low liver copper levels, and was supported by the signalment and clinical presentation. Caprine arthritis and encephalitis virus (CAEV) was initially considered as a differential diagnosis, but the lack of inflammatory reaction within the nervous tissue suggested that it was not involved in the development of these lesions. The pulmonary edema observed in all four goats was most likely secondary to the euthanasia.

Enzootic ataxia, also known as delayed swayback, is a copper deficiency myelopathy[1]. Swayback is a congenital form of the disease that affects neonates; this was not observed in this case. Ataxic kids are normal at birth, but develop clinical signs between one week and six months of age.[2,4] There are differences in the metabolism of copper in sheep and goat, and between breeds within species.[6] The delayed form of swayback is frequently seen in goats, while the congenital form is rare in this species.[1,3] The Angora and Boer breeds of goats have been suggested as being particularly susceptible to low copper levels.[1,4,5]

The disease is thought to be caused by a deficiency in the intake of copper by the dam during pregnancy.[1] Most commonly this is a primary deficiency due to inadequate intake from the diet, but a secondary form can occur where dietary molybdenum, zinc and cadmium impair the absorption of copper.[1,6] The exact diet of the does during pregnancy in this case is unknown, but reportedly no mineral supplementation was provided. In this case, all four kids had liver molybdenum and zinc levels within the normal ranges while cadmium levels were low. In addition to the copper deficiency, all goats showed low levels of selenium and cadmium.

The exact pathogenesis of enzootic ataxia remains unclear, mainly due to the unknown mechanism of action of copper in the developing nervous system.[3,6] Albeit, copper is required for the activity of several enzymes that are essential for neural

Spinal cord, goat. Neurons (arrows) within the ventral horns are often swollen with dissolution of Nissl substance (central chromatolysis). (HE, 136X)

function, including cytochrome oxidase and superoxide dismutase, amongst others. The effects of copper deficiency on the central nervous system occur in utero and during early neonatal life.[2,3,6] Copper deficiency leads to suppression of mitochondrial respiration and reduced phospholipid synthesis. This energy failure is likely to play a role in the axonal and neuronal degeneration that is observed histologically.[3] The generation of reactive oxygen species (ROS) have also been implicated in these changes. . Despite the aforementioned changes, the animal's ability to metabolize copper is not impaired; therefore, tissue concentrations in affected animals may return to normal after dietary correction.[3]

Gross lesions associated with enzootic ataxia in kids are few and not consistent,[3,6] correlating with the lack of relevant gross findings in this case. Microscopic lesions affect the grey and white matter of both the spinal cord and brainstem. In the spinal cord, the dorsolateral aspect of lateral funiculus and the ventromedial tracts of the ventral funiculi are most commonly affected, as evident in this case. . Goat kids show a particularly high incidence of cerebellar degeneration compared to lambs[3], which was however not observed in the here presented case.

JPC Diagnosis: Spinal cord, white matter, ventral and lateral funiculi: Neuroaxonal degeneration, bilaterally symmetrical, multifocal, moderate, with ventral horn neuronal chromatolysis.

Conference Comment: Copper is an essential element for many cellular functions: antioxidant activity (superoxide dismutase), mitochondrial respiration (cytochrome oxidase), catecholamine synthesis (dopamine β-hydroxylase), melanin synthesis (tyrosinase), and iron hemostasis (ceruloplasmin). Copper deficiency causes disease in lambs, goat kids, and piglets and manifests as either absolute primary due to dietary deficiency, or conditioned secondary (most common) due to reduced intestinal absorption, reduced tissue availability, or enhanced secretion. There are several minerals that act as antagonists to copper including: molybdenum, sulfate, iron, and zinc. Ruminants are specifically affected by molybdenum and sulfate which limit copper absorption by forming complexes with copper in the rumen called thiomolybdates. Iron mechanism of antagonism is unknown.[3,6,8]
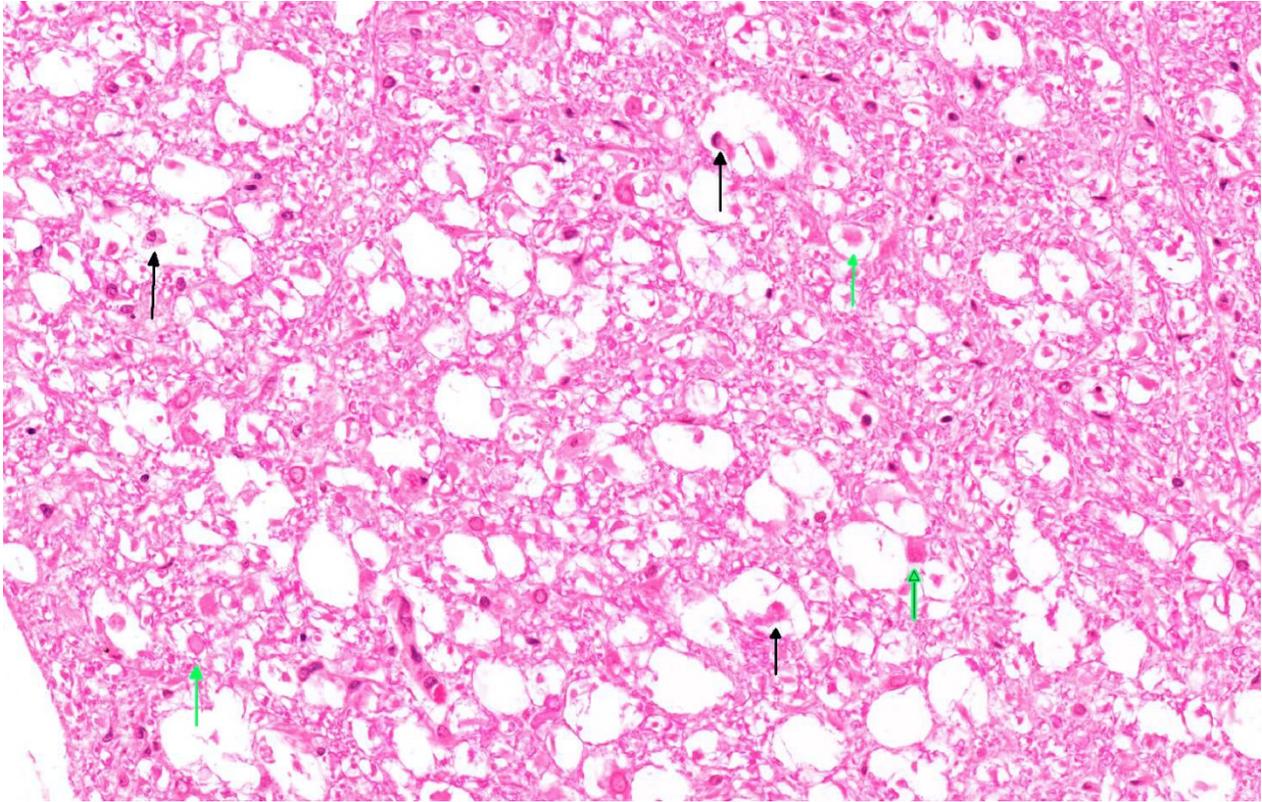
Spinal cord, goat. Within the ventral funiculi, myelin sheaths are markedly dilated and contain swollen axons (green arrows), and gitter cells (black arrows). (HE, 136X)

Clinically, there are two forms of copper deficiency in lambs and goat kids: swayback which is congenital and mainly affects lambs and enzootic ataxia which has a delayed onset and affects lambs between 1 week and several months of age. Affected animals develop progressive neurologic signs such as swaying, falling, spastic paralysis or ascending hindlimb paralysis[7], ataxia, blindness, or deafness. Grossly, only lambs with congenital swayback have lesions that are apparent in the cerebral white matter, evident as bilaterally symmetrical cavitation within the occipital pole or entire corpus medullare. Lambs with delayed enzootic ataxia may develop lesions at any part of the neuraxis in the gray or white matter, but those lesions have not been clearly defined. In both forms, the microscopic changes in the spinal cord are the most characteristic and consist with Wallerian degeneration in the dorsolateral and ventromedial tracts throughout the spinal cord, a pattern suggestive of a distal axonopathy. Additionally, there are degenerative neuronal changes within the red, lateral vestibular, medullary reticular, and dorsal spinocerebellar nuclei in Clarke's column, and in the spinal motor neurons of the intumescences.[3,6,8]

The pathogenesis for these syndromes is not fully understood, but most likely represents the culmination of numerous factors to include energy failure from altered function of mitochondrial cytochrome oxidase and subsequent neuronal degeneration and/or inadequate function of copper-zinc superoxide dismutase leading to oxidative damage.[3,6,8]

In piglets, many of the white matter changes associated with copper deficiency are similar to kids and lambs; however, there are additional neuronal changes. They also can develop skeletal and elastin abnormalities leading to fractures and arterial rupture.[3,6,8] In addition to the neurological manifestations of copper deficiency, sheep also develop "steely wool" (wiry, poor hair coat), hypopigmentation of black wool, and osteoporosis. Poor hair coat and achromotrichia are particularly prominent in cattle which may develop pale colored hair around their eyes, called "spectacles".

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CASE IV: 2 (white colored slide) (JPC 4065579).

Signalment: 1.5-year-old, female, *Cavia porcellus*, guinea pig.

History: The animal was a pet that developed severe abdominal enlargement. At surgery one of the ovaries was massively enlarged (approximately 6X4 cm).

Gross Pathology: None provided.

Laboratory results: None provided.

Microscopic Description: Almost 100% of the ovary (part of the tumor submitted) is replaced by an irregularly round, unencapsulated, poorly demarcated, densely cellular, pleomorphic, expansile neoplasm that extends to the cut borders of the sample provided. (The lesion was delimited by the ovarian capsule).

Neoplasm is composed of variably arranged cells with a derivation from both mature and embryonal elements of all three primordial germ cell layers, embedded in a variable amount of fibrovascular stroma.

Approximately 60% of the neoplasm consists of neuroectodermal neoplastic cells that multifocally palisade around either central areas composed of brightly
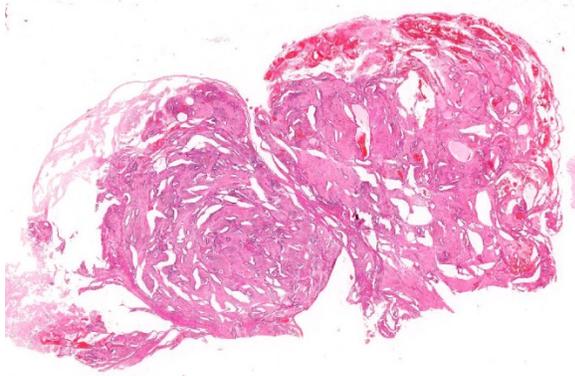
Ovary, guinea pig. The ovary is replaced by an expansile, multilobular, cystic neoplasm. (HE, 4X)

eosinophilic, homogeneous protein-rich material (neuroepithelial rosettes mimicking ventricular spaces and ependyma) surrounded by sheets of glial cells (mimicking glial production zone) or around blood vessels (pseudo-rosettes). Neoplastic cells are oval to cylindrical, 15-20 micron in diameter, have indistinct borders, high N/C ratio and central or palisading hyperchromatic oval nuclei with indistinct nucleoli. There are scattered larger (30-40 micron in diameter), polygonal to stellate cells with abundant, pale basophilic, granular cytoplasm and a central round nucleus with single prominent nucleolus (well differentiated neurons) embedded in an abundant, pale eosinophilic, vaguely fibrillar to vacuolated background substance (neuropil).

Ectoderm is represented by few tubules and cystic spaces lined by single or multiple layers of cuboidal to columnar, 20-24 micron in diameter, epithelial cells with distinct cell borders, polarized nuclei and evident apical capitation of the cytoplasm (consistent with apocrine gland origin).

Mesodermal elements include bundles of fusiform cells 30-35 micron in length with indistinct cell borders, abundant pale eosinophilic cytoplasm and central, cigar shaped nuclei (well differentiated smooth muscle tissue), multiple focal islands of oval to stellate cells loosely embedded in abundant pale basophilic, myxoid matrix (consistent with myxoid to embryonal cartilage- not included in all sections) and rare, small multifocal areas of mineralized lamellar bone with intralacunar round to oval osteocytes (not included in all sections).

Endoderm comprises multiple, variably sized, cystic spaced, up to 0,5 mm in diameter, filled with variable amounts of amphophilic, fibrillar to granular material (mucus), lined by pseudostratified epithelium composed of columnar to bottle-shaped cells, with basal to centrally located nucleus and apical ciliated border (consisting of respiratory epithelium) or few acini and tubules of epithelial polygonal cells with pale eosinophilic cytoplasm expanded by secretory pale vacuoles and flattened basal nuclei (consisting with salivary or goblet cells).

Cellular atypia such as anisocytosis anisokaryosis are mild in all cell populations; occasional multinucleated, undifferentiated neoplastic elements are seen; mitoses are fewer than 1 per 10 HPF.
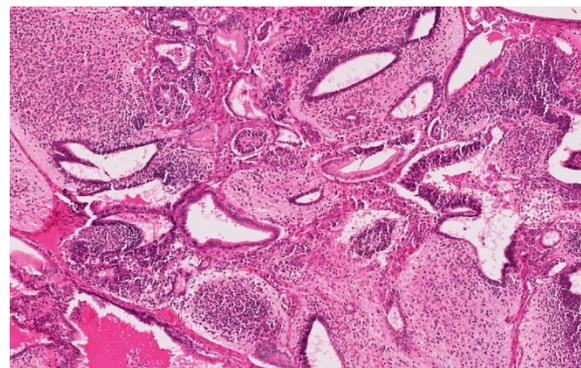

Ovary, guinea pig. At higher magnification, the mass is largely composed of neural tissue with condensation of primitive neuroepithelium forming ependymal-like rosettes. (HE, 80X)

Almost 25% of the mass is composed of elevated numbers of extravasated erythrocytes (hemorrhages) admixed with a meshwork of fibrillar lightly eosinophilic extracellular material (fibrin) and foci of pale eosinophilic, granular to amorphous extracellular material mixed to karyorrhectic cell debris (liquefactive necrosis).

Contributor's Morphologic Diagnosis: Ovary, ovarian teratoma, guinea pig (*Cavia porcellus*)

Contributor's Comment: Teratomas are complex neoplasms composed of tissues representative of at least two, (sometimes all three) primordial germinal layers that are the ectoderm (nervous tissue skin including adnexa), mesoderm (connective tissue, muscle, bone, cartilage, and urogenital and cardiovascular system), and endoderm (gastrointestinal and respiratory epithelium, including their glandular structures).[9] Teratomas are tumors considered to emerge from proliferation of totipotent stem cells occurring physiologically in gonads and that sometimes may reside in abnormal location due to anomalous migration or lack of regression of midline embryonic rest. Such cells have the capacity to differentiate into any of the cells types of the adult body.[6] Teratomas usually occur in the gonads[9,16]
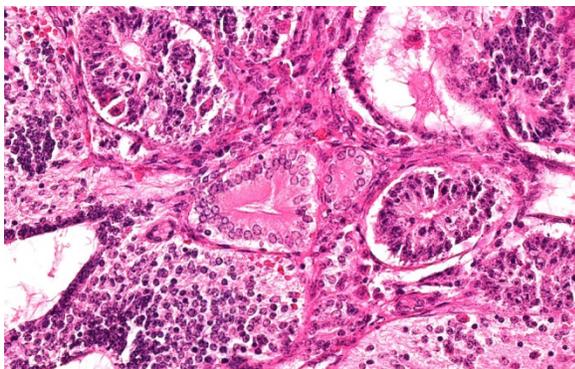
Ovary, guinea pig. Scattered among the ependymal rosettes are nests of ciliated columnar epithelium resembling respiratory epithelium. (HE, 324X)

and more frequently in the ovary where they may cause spherical to ovoid severe enlargement with a gross aspect of solid and cystic areas on cut surface[9] but reports about extragonadal teratomas, involving cutaneous structures, the alimentary tract, the kidneys, and retroperitoneal and retrobulbar spaces, as well as other systems, also exist.

Teratomas are considered uncommon in domestic animals[9,16] and have been reported in humans, nonhuman primates, dog, cat, horse (base of the ear), sheep, ox, rabbit, swine, laboratory rodents, ferret (adrenal gland), poultry, a blue heron, frogs, hares, squirrel, a spot-necked otter, a woodchuck, turtles, porpoise, hedgehog, red-eared slider and a giraffe.[4,10,11,12,15,17,20] In guinea pigs, primary tumors of the reproductive tract represent approximately 25% of spontaneous tumors.[13] Among these, teratoma is the most frequent.[19,21] Differential diagnosis in guinea pigs include cystic rete ovarii seen commonly in older sows.[13] Teratomas in mice have been rarely reported in B6C3F1, LT/Sv, CD1, C3H strains, with the exception of inbred strain 129 mice. Indeed, approximately 1/3 of 129 teratoma substrain male mice develop spontaneous testicular teratomas.[14] An ovarian teratoma with associated trisomy of chromosome 16 has been reported in a baboon.[10]

Classification of a teratoma as benign or malignant is based largely on the relative amount of primitive, undifferentiated cell content and type of tissues within the tumor: when all the components are well differentiated they are classified as benign (mature) teratomas; on the contrary, if undifferentiated cells/tissue are predominant, and immature neuro-ectodermal elements are over-represented, the tumor is termed teratocarcinoma and is considered malignant.[6] In general, the

presence of undifferentiated cells aggravates the prognosis, and teratomas with incompletely differentiated tissues should be considered potentially malignant.[7,16]

However, in the present case, despite the major composition by variably differentiated neuro-ectodermal tissue and the complete substitution of the ovarian parenchyma, a final diagnosis of teratoma and not of teratocarcinoma was made, due to the presence of several well differentiated and recognizable cell lines and absence of neoplastic emboli or distant metastasis. In animals most ovarian and prepuberal testicular teratomas are considered benign, whereas most post pubertal testicular tumors in men are malignant, suggesting a differential origin from benign and malignant cells respectively.[8] The difference may reside in the human tolerance for parthenogenetic development of immature somatic ovarian cells into three germ layers while suppressing neoplastic cells, in contrast to the human male that differentiates malignant immature somatic cells less efficiently in the embryo.[18] The k-FGF gene, a member of the family of fibroblast growth factor genes, is considered as a marker for murine malignant, testicular, teratoma.[5]
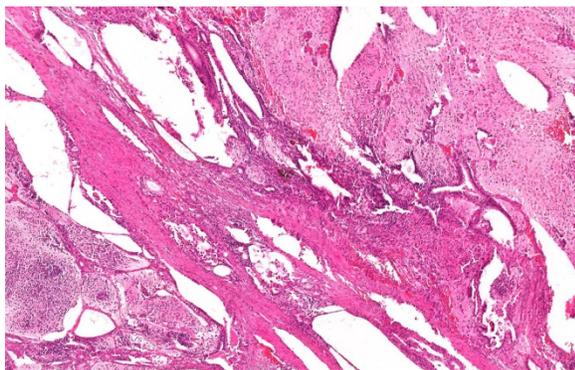

Ovary, guinea pig: Dense bands of smooth muscles course through the neoplasm. (HE, 288X)

JPC Diagnosis: Ovary: Teratoma, *Cavia porcellus*, guinea pig.

Conference Comment: As an excellent review of teratomas is provided by the contributor, our conference comment will focus on ovarian tumors in general.

Ovarian tumors are classified based on embryologic origin: (1) epithelial (surface epithelium of modified mesoderm, rete ovarii, or subsurface epithelial structures
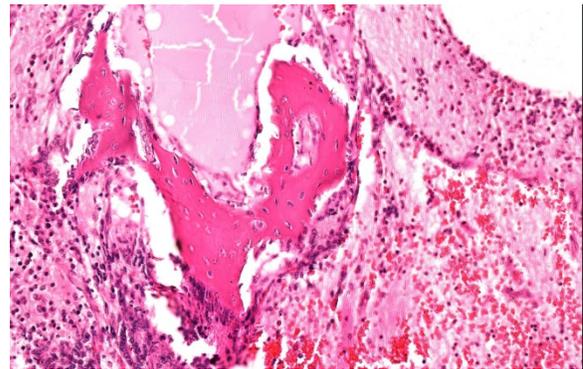
Ovary, guinea pig. Spicules of well-differentiated bone are scattered throughout the neoplasm. (HE, 196X).

(SES, in bitches); (2) germ cells; (3) ovarian stroma (sex cord stromal or gonadostromal). More specifically, sex cord stromal elements are the theca and granulosa cells and their luteinized derivatives which are essential to the formation of primary follicles.[1] A review of ovarian follicle progression is prudent at this juncture. Primordial follicles (the most immature) are plentiful in the adult ovary located deep to the tunica albuginea and composed of a central oocyte surrounded by a layer of simple squamous follicle cells. Hormonal stimulation induces maturation to a primary follicle composed of the central oocyte which is developing, surrounded by a layer of cuboidal cells which proliferate to form a multilaminar (late primary) follicle. An antrum progressively forms as the follicle cells conjoin and leave an open fluid filled space as the zona pellucida forms around the outside of the follicle. The

follicle is termed "secondary" once the antrum has widened into a C-shape. At this point, the follicle cells are called membrana granulosa and a layer of stromal cells form around the outer zona pellucida called the theca folliculi. The theca folliculi has two layers: the interna (a more cellular, inner vascular layer) and the externa (an outer layer of connective tissue). Finally, continued stimulation and growth leads to Graafian (tertiary) follicle formation in which the oocyte is surrounded by several layers of membrana granulosa cells auspiciously termed the cumulus oophorus. Following ovulation, granulosa and theca interna cells multiply, hypertrophy, and differentiate into granulosa and theca lutein cells which form the corpus luteum and produce progesterone. Assuming there is no pregnancy, the corpus luteum regresses during the end of diestrus to form a corpus albicans followed by atresia and resorption of the remaining structure.[2]

Ovarian epithelial tumors are most common in the bitch, arising from the SES, and tend to form cystic nodules that elevate the surface of the ovary. Metastasis of carcinomas occurs via implantation with ascites. Sex cord stromal tumors, on the other hand, are more common in the mare, cow, and queen and are named according to the originating cell type of the ovarian endocrine scheme (granulosa cell tumor, granulosa-theca cell tumor, thecoma, luteoma, Sertoli cell tumor of the ovary or lipid cell tumor). These tumors, being endocrine in origin, frequently produce hormones such as progesterone, estrogen, or inhibin resulting in systemic effects like anestrus, persistent estrus (nymphomania), masculinization, and blood dyscrasias. Granulosa cell tumors are the most common sex cord stromal tumor and are composed of aggregates of granulosa cells with the vague appearance of follicle formation surrounded and separated by a supporting stroma of spindle cells. Some tumors (more frequent in horses) contain Cal-Exner bodies, homogenous eosinophilic deposits within the center of the follicular structures, which are a useful microscopic feature. In dogs, granulosa cell tumors may appear to be composed of Sertoli cells. For that reason, this type is called Sertoli cell tumor of the ovary. Finally, germ cell tumors of the ovary can be broken down into two main categories: dysgerminoma or teratoma. Dysgerminomas are extremely rare in domestic animals and are composed of broad sheets of large cells with prominent nuclei and scant cytoplasm. Grossly, they are large, grey to white, and firm. Teratomas (described in detail above) are, by definition, composed of two or more germinal layers.[1] The germ cell layers are ectoderm, mesoderm, and endoderm and form the components listed below.

Table 1: Germ cell layer derivatives[3]

Ectoderm	Mesoderm	Endoderm
Epidermis of skin and its derivatives (sweat glands, hair follicles)	Notochord	Epithelial lining of digestive tract
Epithelial lining of mouth and anus	Skeletal system	Epithelial lining of respiratory system
Cornea and lens of eye	Muscular system	Lining of urethra, urinary bladder, and reproductive system
Nervous system	Muscular layer of stomach and intestine	Liver
Sensory receptors in epidermis	Excretory system	Pancreas
Adrenal medulla	Circulatory and lymphatic systems	Thymus
Tooth enamel	Reproductive system (except germ cells)	Thyroid and parathyroid glands
Epithelium of pineal and pituitary glands	Dermis of skin	
	Adrenal cortex	

Conference attendees discussed the potential locations for this tumor because there was no apparent normal tissue present. One participant pointed out that even within completely effaced ovaries, there is at usually SES and surface epithelium remaining. It is unclear whether SES are present in guinea pig ovaries since available resources only confirmed their presence in canine ovaries.[2,13] However, in some sections there were remnants of surface epithelium suggesting the submitted slide was truly ovarian in nature.

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