CASE I – 03-0975 (AFIP 2889972)

Signalement: 1.5-year-old, female, Jack Russell terrier, canine.

History: The dog presented to the Small Animal Clinic because of seizure activity for the previous 1.5 hours. Upon presentation the dog was immediately admitted to the Intensive Care Unit (ICU). At admission to the ICU the animal was in respiratory and cardiac arrest. Resuscitation efforts were initially successful but the dog soon arrested again and died.

Forty-five days prior to presentation to the Small Animal Clinic the dog had whelped. The bitch had been nursing her puppies and had been presented to the referring veterinarian once for hypocalcemia.

Gross Pathology: Both horns of the uterus were dilated to a diameter of 2 cm and the lumen of both contained a moderate amount of mucoid, red-brown material. Scattered along the length of both uterine horns were multifocal, mural, ellipsoidal enlargements that were approximately 4 cm in length and bulged into the uterine lumen. The mucosal surface of these enlargements was red-brown and covered with a friable, necrotic tissue and the mucoid material described above.

Laboratory Results: None reported.

Contributor’s Morphologic Diagnosis: Uterus: Endometrial ulceration, necrosis and hemorrhage, diffuse, severe, with invasion by placental trophoblast-like cells (subinvolution of placental sites), Jack Russell terrier, canine.
Contributor’s Comment: This animal died of acute respiratory and cardiac arrest most likely secondary to prolonged muscle rigidity (seizures). The cause of the muscle rigidity is believed to be hypocalcemia related to nursing (eclampsia). The uterine lesions are not related to the death of the dog.

Histologically, the uterine lesions consist of an irregular, ulcerated luminal surface covered with eosinophilic debris, collagen and erythrocytes. Subjacent to the eosinophilic debris are degenerating placental trophoblast-like cells that have eosinophilic fragmented cytoplasm. There are multifocal areas of hemorrhage scattered about within the eosinophilic debris, collagen and degenerating trophoblast-like cells. Scattered randomly within the lesion are moderate numbers of macrophages many of which contains a brownish granular pigment (hemosiderin). Multifocally covering and randomly distributed within the eosinophilic debris and degenerating trophoblast-like cells are small sheets to islands of viable placental trophoblast-like cells. These epithelial cells have mildly distinct cytoplasmic borders and variable amounts of finely to moderately vacuolated eosinophilic cytoplasm. Nuclei are oval to irregular, moderately basophilic and have finely clumped randomly distributed chromatin. Nucleoli are small to moderate in size, moderately basophilic and randomly placed within the nucleus. Villous projections lined by highly vacuolated cuboidal to columnar epithelial cells are prominent in some fields. Deep to the degenerating and viable placental epithelium are scattered normal uterine glands. There are small to moderate numbers of lymphocytes and macrophages scattered between the normal endometrial glands. There are numerous congested vessels and scattered vessels contain fibrin thrombi. The myometrium is moderately hypercellular.

Subinvolution of placental sites was first described as a clinical and pathological condition in 1966. Subinvolution of placental sites is an important differential diagnosis in the postpartum bitch that has a persistent bloody uterine and vaginal discharge. In the normal postpartum bitch, uterine hemorrhage usually ceases within 1 to 2 weeks following parturition. Uterine bleeding in dogs with subinvolution of placenta sites (SIPS) can continue well into the postpartum period sometimes lasting up to 7 to 12 weeks following whelping. There has been one reported case where a dog with SIPS spontaneously recovered. The condition is usually treated with an ovariohysterectomy.

The cause of SIPS is unknown and the condition has only been recognized in the dog. Several theories (reviewed in reference 5) have been proposed for the condition but none has received wide acceptance. Early theories proposed the presence of bacterial infections in the placental implantation sites that prevented normal uterine involution. Another theory proposes that the condition is caused by an imbalance in estrogen and/or progesterone. A more recent proposal suggests
that there may be a failure of normal endometrial blood vessel thrombosis following parturition. In this theory the lack of normal thrombus formation is attributed to vascular damage caused by trophoblast-like cell migration along uterine blood vessels.

The histological appearances of normal postpartum involution\textsuperscript{2} and subinvolution of placental sites\textsuperscript{1} have both been described. Histologically, both lesions are similar but in dogs with subinvolution of placental sites the uterine lesions persist for longer than normal.

**AFIP Diagnosis:** Uterus: Subinvolution of placental site, Jack Russell terrier, canine.

**Conference Comment:** Typical gross findings in cases of subinvolution of placental sites are multiple ellipsoidal enlargements of the endometrium visible from the serosal surface. These segmental thickenings are sites of previous placental attachment and the mucosal surface is characterized by hemorrhagic, irregularly thickened, rough, gray to brown plaques up to twice the size of a normal placental site from the same breed at the same stage after parturition. The endometrium between the sites is normal.\textsuperscript{2,7-10}

The key histologic finding is the presence of syncytial masses of trophoblast-like cells in the endometrium, often surrounding blood vessels. These cells invade the myometrium and, in some cases, may perforate the serosa. Other characteristic histologic findings include a plaque that protrudes into the uterine lumen composed superficially of necrotic debris and regenerating endometrium. Deeper within the plaque there is collagen deposition, hemorrhage, and dilated endometrial glands.\textsuperscript{2,7-10}

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**References:**

**CASE II – 03N0808 (AFIP 2890687)**

**Signalment:** Near term (one-month premature by breeding dates and crown-to-rump length), female Morgan fetus.

**History:** Fetus and intact amnion were found on the ground in the pasture one-month prior to term. The mare, a 6 year-old Morgan mare, showed no systemic illness and was current on all vaccinations. The mare had foaled once before with no complications.

**Gross Pathology:** The villous surfaces of the gravid and non-gravid placental horns were diffusely red to dark red with multiple, randomly distributed avillous foci up to 0.3cm in diameter. Attached to the umbilicus were multiple yolk sac remnants. There were multiple epicardial and endocardial petechial and ecchymotic hemorrhages that were most pronounced in the left ventricle. Petechial and ecchymotic hemorrhages were also noted in the left caudal lung lobe and liver.

**Laboratory Results:** Bacterial cultures of the fetal liver were negative.
**Contributor’s Morphologic Diagnosis:** Placenta: Multifocal nonsuppurative placentitis with necrosis and abundant intralesional *Encephalitozoon* sp.

**Contributor’s Comment:** Scattered throughout the chorioallantoic villi there are multifocal to coalescing foci of necrosis of the chorionic epithelium. (Fig. 1) Shed necrotic chorionic epithelial cells are frequently noted. (Figs. 2 and 3) Associated with foci of necrosis, as well as within unaffected regions, there are abundant numbers of individual or aggregates of protozoa both free and within chorionic epithelial cells. These organisms are oval in shape and measure approximately 1-2um. (Fig. 4) These organisms are variably positive when stained with the following histochemical stains: Gram stain, silver (Steiner’s) stain, acid-fast stain and Giemsa stain. Ultrastructurally, organisms were identified as spores of the phylum Microspora based upon spore size, cross sections of a coiled polar filament, the presence of a thick wall and posterior vacuole, and by the presence of organisms within typical parasitophorous vacuoles. (Figs. 5 and 6) Based upon these findings a diagnosis of encephalitozoonosis was made.

*Encephalitozoon cuniculi* is a protozoan of the Phylum Microspora. Recent molecular analyses of microsporidia have suggested a closer relationship to fungi rather than protozoa based upon the presence of a mitochondrial heat shock protein and alpha- and beta-tubulins that closely resemble those of fungi, as well as the presence of chitin and trehalose, both of which are components of fungi. The organism is an obligate intracellular parasite and is most commonly recognized as a cause of nephritis and encephalitis in rabbits. *Encephalitozoon cuniculi* infections have also been described in birds, mice, rats, guinea pigs, hamsters, cats, dogs, wild carnivores, humans, and non-human primates. Several species of *Encephalitozoon* have been implicated as causes of disease in immunocompromised people, and encephalitozoonosis has become an issue of increased concern amongst AIDS patients.

In most species infection is usually subclinical and organisms are found incidentally. When present, lesions in the brain usually consist of small granulomas located most commonly in the cerebral cortex. In more severe cases large areas of necrosis and lymphocytic perivascular cuffing are often present. Renal lesions are usually characterized by granulomas and lymphocytic infiltrates involving the renal tubules and interstitium. In addition, the organism has been associated with lesions in the liver, pancreas, adrenal, spleen and lung. In blue foxes the organism has also been associated with the development of polyarteritis nodosa, and appears to be a lesion unique to this species.

Infection generally occurs by ingestion of spores. Once ingested, the spores are able to inject their sporoplasm, via the extruded polar filament, into an appropriate host cell. Once inside the host cell the sporoplasm undergoes asexual
proliferation (merogony) with the formation of meronts. Meronts undergo differentiation into sporoblasts (sporogony) and eventually develop into spores, which are packaged within a parasitophorous vacuole. Enlargement of the parasitophorous vacuole eventually leads to cell rupture and release of spores into extracellular spaces. Dissemination can occur by both direct extension into surrounding cells, or by introduction into the vascular system. Spores are typically shed in feces, urine and mucus.\textsuperscript{1,9,10}

Both horizontal and vertical transmission of encephalitozoonosis occurs. Transplacental infections have been documented in numerous species, and lateral transmission among young within the same group is known to occur.\textsuperscript{4} Placental lesions are uncommon findings in most species with encephalitozoonosis and have been reported in a single squirrel monkey and a Quarterhorse and consisted of granulomatous placentitis and necrotizing placentitis respectively.\textsuperscript{11,12}

A number of methods for antemortem diagnosis of encephalitozoonosis have been described. Most common methods include detection of specific antibodies by IFA, ELISA, CIA, and serology. PCR has also been utilized for diagnosis.\textsuperscript{13} Postmortem diagnosis is based upon the finding of characteristic histologic lesions and/or the demonstration of organisms within tissues. A variety of staining methods can be used to demonstrate microsporidia in formalin-fixed and paraffin-embedded tissues. Mature spores usually stain Gram-positive utilizing the Brown and Brenn method of tissue Gram staining. Silver staining, such as the Warthin-Starry method, is another useful histochemical means of identifying the organism, and will identify both mature and developing stages of microsporidia. Other histochemical methods such as the periodic acid-Schiff stain can be used, but are not optimal for the identification of organism within tissues.

Electron microscopy is considered the gold standard for diagnostic conformation and species identification. Electron micrographs reveal organisms within parasitophorous vacuoles and a distinctive polar filament measuring approximately 100-150nm in diameter. Spores measure approximately 2\textmu m in length and enclose an extrusion apparatus that consists of the polar filament, an anchoring disc (polar sac) and a complex stack of membranes known as the polaroplast. The posterior vacuole and cytoplasm occupy the remaining space within the spore wall.\textsuperscript{2,3}

AFIP Diagnosis: Chorioallantois: Degeneration and necrosis, multifocal to coalescing, marked, with loss of chorionic villi, mild subacute inflammation, and intratrophoblastic microsporidia, Morgan, equine.
Conference Comment: The contributor gives a comprehensive overview of encephalitozoonosis. In addition to the changes noted by the contributor, conference attendees identified squamous metaplasia of trophoblasts.

It is difficult to differentiate *Encephalitozoon cuniculi* by light microscopy from other small protozoan parasites, especially *Toxoplasma gondii*. All stages of microspora are gram positive and the spores are birefringent, both of which differentiate *Encephalitozoon* from *Toxoplasma*.\(^3\) The birefringent property of the spores of microsporidia is a result of chitin in the endospore layer.\(^13\) Cysts of *T. gondii* are smaller (60um diameter) than the pseudocysts of *E. cuniculi* (60-120um diameter), and mature *Toxoplasma* organisms are larger (2 x 6 um) than those of *Encephalitozoon* (1.5 x 2.5um).\(^12\)

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References:

**CASE III – CASE 1 (AFIP 2890505)**

**Signalment:** 2-year-old CD1 female mouse.

**History:** This control (untreated) mouse on a 2-year study was euthanized. Clinical signs included abdominal distension, pale appearance, blood in the cage pan, red vaginal discharge, staining of fur and perineal staining.

**Gross Pathology:** The uterus was enlarged bilaterally with multiple nodular foci in both horns that were clear, red or yellow. There were bilateral ovarian cysts, the left was clear and 10x12 mm, while the right was dark and 5x5mm.

**Laboratory Results:** None reported.

**Contributor’s Morphologic Diagnoses:** Decidual reaction affecting the endometrium and myometrium. Additional findings: angiectasis, thrombosis, cystic endometrial hyperplasia, abscess.

**Contributor’s Comment:** Elderly female CD1 mice have a high incidence of cystic endometrial hyperplasia and often develop angiectasis and sometimes thrombosis in the uterus, but decidual reactions are rare. Decidual reactions, sometimes referred to as deciduomas, are seen occasionally in mice and rats, often in younger animals than this case, and can present a diagnostic challenge in the differentiation from neoplasms. Decidual reactions occur in the endometrium, but in this case there is also extensive proliferation within the adjacent myometrium and this could be confused with a smooth muscle neoplasm. This proliferation in the myometrium resembles the submucosal proliferative lesion of the trigone of the urinary bladder.
of male mice. The amount of this proliferative tissue varies between slides but some is present in both the endometrium and the myometrium on all slides.

The clinical signs relating to blood loss and vaginal bleeding were considered to be due to angiectasis, thrombosis, cystic endometrial hyperplasia and abscess.

AFIP Diagnoses:
1. Uterus: Decidual reaction with myometrial infiltration, CD1 mouse, rodent.
2. Uterus: Endometritis, neutrophilic, acute, diffuse, moderate, with intraluminal bacteria.

Conference Comment: Conference attendees discussed the presence of an apparently discrete extramyometrial nodule present in some slides. It was concluded that, due to the similarity in organization and histologic characteristics (to the decidual reaction), that this is part of the same process and extends into the myometrium and serosa. We consider the bacterial endometritis to be secondary to the decidual reaction.

In addition to deciduomas, other proliferative lesions of the mouse uterus were discussed. Histiocytic sarcomas may involve multiple tissues, although a common site is the uterine wall. It is not clear if the uterus is the primary site but since the liver is invariably involved, hepatic origin has been proposed. Histiocytic sarcoma in the uterus should be distinguished from schwannomas or poorly differentiated leiomyosarcomas. Endometrial stromal sarcomas are common and may arise within endometrial stromal polyps or are found in the uterine wall. Endometrial adenomas and adenocarcinomas arise from the epithelial lining of the uterine mucosa or endometrial glands.

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References:
CASE IV – E 2228/02 (AFIP 2885741)

Signalment: Dog (*Canis familiaris*) Picard, 10 months old.

History: When acquired, the dog was thought to be female. Later on, as it matured, the animal indicated discomfort whenever it sat down, and a prolapse of a penis-like mass from the vulva was observed. Veterinary examination revealed a "penis-anlage" in the fossa clitoridis (praeputialis) of the vulva. Surgical excision of this "anlage" and of the internal genitalia (designated as uterus and testes by the referring veterinarian) was performed. The excised tissues were placed in 7% formaldehyde and passed on for pathological examination.

Gross Pathology:
1. Clitoris: large (4 x 1 x 1 cm), with a knobby apical swelling, and a massive central os clitoridis.
2. Uterus: with two inconspicuous uterine horns (0.4 x 0.4 x 10.5 cm each) and a corpus uteri.
3. Gonads: testis-like organs (1.5 x 0.7 x 2 cm each)

Laboratory Results: None reported.

Contributor’s Morphologic Diagnosis: Gonads: Bilateral ovotestis (true, bilateral hermaphroditism), Picard, canine.

Contributor’s Comment: Originally, the case had been diagnosed as unilateral ovotestis in conjunction with contralateral hypoplastic testis. But during production of slides for AFIP Wednesday Slide Conference, deeper sections of the gonadal tissue became accessible for histologic examination. In a great number of slides an ovotestis and an apparent hypoplastic testis can be seen: Ovotestis consists of a peripheral zone of ovarian tissue, in which the complete sequence of follicular maturation (primordial follicle up to the tertiary follicle) can be discerned, and a central zone of testicular tissue, where both interstitial cells and hypoplastic seminiferous tubules lined by Sertoli cells can be observed. The contralateral testis exclusively shows testicular tissue with hypoplastic seminiferous tubules and normal appearing interstitial cells. Moreover parts of a hypoplastic epididymic duct and ductus deferens, with attenuated epithelium each, are visible. However, in deeper sections of the gonad that had originally been designated as hypoplastic...
testis, a peripheral zone of ovarian tissue becomes evident, too. The uterus (not present in the sections) shows normal tissue.

Hermaphrodites have ambiguous genitalia with part or all of the genital organs of both sexes present. The intersexual condition is subclassified into true (hermaphroditismus verus) and pseudohermaphroditism, the distinction being based on the presence of both types of gonadal tissue in the true hermaphrodite. The pseudohermaphrodite has gonads of one sex and accessory reproductive organs of the opposite sex. The true hermaphrodite either appears as unilateral (present case: testicular and ovarian tissue on one side, testicular or ovarian tissue on the other side), bilateral (testicular and ovarian tissue on both sides), or lateral (testicular tissue on one side, ovarian tissue on the other side). Germinocytes can only be found in the ovaries and in the ovarial parts of ovotestes. The accessory reproductive organs differ as the case may be, and can develop in varying degrees into male or female direction during ontogenesis.

True hermaphroditism has only rarely been observed in domestic animals, occurring most often in swine. But it has been described in goats, dogs, cats and horses, too. The development of this malformation is poorly understood. Physiologically, embryonal development of the genital ridge either into an ovary or a testis depends on the absence or presence of an intact Y-chromosome. For the testicular differentiation, the SRY-gene (Sex Determining Region on the Y-Chromosome) is essential. It is responsible for the induction of testicular development, and the Sertoli cells to produce Müllerian Inhibiting Substance (MIS), leading to Müllerian duct-degeneration. Zygogenetic investigations have shown that in humans 80% of the hermaphrodites show a female karyotype 46, XX (a small number of patients shows 46, XX/46, XY mosaicism, and a very small number 46, XY). The development of testicular tissue in the absence of a Y-chromosome contradicts the above cited theory that a genetically active Y-chromosome is essential for gonadal differentiation in male direction. Various mechanisms including translocated Y-chromosomal sequences (SRY) or a mutation that allows testis determination without SRY have been discussed.

AFIP Diagnosis: Gonad: Ovotestes, Picard, canine.

Conference Comment: The contributor gives a concise overview of true hermaphroditism and pseudohermaphroditism. Another well-described anomaly of development is that of freemartinism. This is an abnormality of chromosomal sex, in contrast to true hermaphroditism and pseudohermaphroditism which are abnormalities of gonadal sex and phenotypic sex, respectively.
Freemartinism is primarily described in cattle and, although rare, it also occurs in sheep, goats, and swine. A freemartin is a female born as a co-twin to a male and is an XX/XY chimera. The freemartin is sterile because anastomoses between the placental circulations allow androgenic hormones from the male fetus to influence the female fetus. This suppresses female genital development and allows male vestiges to develop. Common gross findings in freemartins are vestigial seminal vesicles (always present), stunted ovaries, a hypoplastic vagina, lack of communication between the vagina and uterus, and an enlarged clitoris.\textsuperscript{6,8}

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**References:**

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