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CASE I – 93196-8 (AFIP 2789818)

Signalment: 1.5-year-old, female, Beagle (Canis familiaris)

History: Untreated control dogs maintained in a barrier facility.

Gross Pathology: None.

Laboratory Results: None.

Contributor's Morphologic Diagnosis: Ovotestes, Etiology unknown.

Contributor's Comment: Disorders of genital development occur in all domestic species, but are rare. They are caused by abnormalities of genetic or chromosomal origin or inappropriate hormone exposure. The mechanisms involved in the production of many abnormalities are not known and a condition cannot often be classified precisely.

Hermaphroditism is subclassified into true or pseudo. The pseudohermaphrodite has only a single type of gonadal tissue. An individual is classified as either male or female pseudohermaphrodite on the basis of the gonadal tissue present. True hermaphrodites have both ovarian and testicular tissue either in the form of a combined gonad (ovotestis) or as separate organs. On an anatomical basis hermaphroditism can appear as bilateral (testicular and ovarian tissue on both sides), unilateral (testicular and ovarian tissue on one side, testicular or ovarian tissue on the other side), or lateral (testicular tissue on one side, ovarian tissue on the other side). True hermaphroditism has been reported in most domestic species, but occurs more often in swine and goats. In a review of 48 cases of intersexuality in dogs, true hermaphrodites accounted for 25% of the

cases; whereas male pseudohermaphrodites accounted for about 50% of the cases.

The external genitalia of true hermaphrodites are often ambiguous and exhibit varying degrees of male and female differentiation. True hermaphrodites with bilateral ovotestes usually have a normal appearing, but hypoplastic, uterus indicating that ovotestes do not secrete adequate quantities of Müllerian inhibitory substance to cause inhibition of paramesonephric duct development. The gonads of a true hermaphrodite are usually retained in the pelvic cavity.

Microscopically, the testicular portion of an ovotestis is always located in the medulla of the gonad, whereas the ovarian portion comprises the cortex. The distribution in an ovotestis of testicular and ovarian tissues varies with the former being more abundant than the latter. Often the ovarian component occupies only a small area on the surface of the gonad. The ovarian portion of an ovotestis contains oogonia, oocytes, and primary and secondary follicles. The testicular portion consists of hypoplastic seminiferous tubules lined by Sertoli cells and rare germ cells. Rete tubules are common in the hilar region of ovotestes. Interstitial cells are generally present between the hypoplastic seminiferous tubules.

In humans and other species, hermaphrodites have an abnormal combination of female or male sex chromosomes. However, the majority of true hermaphrodites have the XX female genotype, but are positive for the H-Y antigen, a minor histocompatibility antigen normally coded for by a gene located on the Y chromosome of males. By studying a family of American cocker spaniels, Selden et al. proposed that the XX male and XX true hermaphroditism are related conditions, resulting from anomalous inheritance of the H-Y male determining gene. Male-determining H-Y genes not located on the Y chromosome may be abnormally transmitted to female offspring. In females homozygous for the mutant H-Y gene, the level of H-Y antigen was similar to that found in normal control males and the gonads developed as ovotestes or testes. When the gene is heterozygous in females, the level of serologically detectable H-Y antigen is lower than that found in normal males and the gonads develop as normal ovaries. In addition, the occurrence of intersex offspring in consanguineous matings of cocker spaniels, but not in outcrosses of putative carriers to unrelated dogs with no family history of intersexuality, indicates that inheritance is recessive. Apparent recessive inheritance of XX true hermaphroditism has been reported in human families, as well as in goats, and in swine. Although the intersex condition has been shown to be familial in some purebred dogs, it is not known if this condition in mixed breed animals is a new mutation or inherited. Since the discovery of H-Y antigen negative phenotypically normal male mice, the significance of the H-Y antigen as the inducer of testicular differentiation remains controversial. Thus, McLaren, et al. proposed the testis determining factor (Tdy) and the gene rendering animals serologically positive for H-Y antigen are separate entities. Further investigation

will be needed to elucidate the mechanisms and determinants involved in sexual differentiation.

AFIP Diagnoses: 1. Gonad: Ovotestis, Cocker Spaniel, canine.
2. Uterus: Essentially normal tissue (not present in all sections).

Conference Comment: The sex of an individual can be defined by chromosomal, gonadal, or phenotypic characteristics. Chromosomal sex, which is fixed at fertilization, is typically straightforward, most commonly being defined as XX or XY. Gonadal sex and phenotypic sex determination on the other hand, is brought about by a complex interplay between the expression, timing, and level of various testis promoting and suppressing genes as set forth by the chromosomal sex.

Embryologically, the genital ridge has the capacity to develop into either a testis or ovary. Female gonadal and phenotypic development is the default condition, while an intact Y chromosome is necessary for masculine differentiation. Because of its close association with maleness, the H-Y antigen gene was at one time believed to play a role in testis determination. Recent reports suggest that it is not the H-Y antigen gene but the closely associated Sry (*s*ex-determining *r*egion *Y*) gene that is responsible for initiating the cascade of events which lead to testis induction. The Sry gene is Y-linked, encodes a high mobility group (HMG) box (a DNA binding region essential for transcription), and promotes Sertoli cell differentiation. While Sry is thought to be the key, multiple genes contribute to sexual differentiation. In recent studies, Sry negative sex reversal males have been described, suggesting that other autosomal genes may have testis-determining properties. While the roles of many genes are not characterized, some (e.g. Sox9) assist in testis development while others, (e.g. DAX-1) repress transcription and act as "antitestis" genes.

Male phenotypic sex is determined by the presence of the embryonic testis. Sertoli cells of the developing testes secrete Mullerian inhibiting substance causing regression of the paramesonephric duct (Mullerian duct). Testosterone production by Leydig cells prevents regression of the mesonephric duct (Wolffian duct) bringing about formation of the vasa deferentia, seminal vesicles, and epididymides. Testosterone is further converted to dihydrotestosterone, playing an important role in the development and masculinization of external genitalia. Phenotypic abnormalities associated with intersex conditions include: ambiguous genitalia, hypospadia, segmental uterine aplasia, and uterus didelphys.

Contributor: Schering Plough Research Institute, Lafayette, NJ 07848

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CASE II – 01-21068 (AFIP 2789979)

Signalment: 8-year-old, female, Holstein, Bovine

History: This cow is from a dairy herd suffering from widespread endometritis which starts within a week of parturition and is refractory to antibiotic therapy and intra-uterine infusion. Over 25 animals have been affected in the last year and 14 have died with complications associated with pyometra including hypocalcemia, ketosis, and septicemia or septic abscesses. This cow developed a uterine discharge at around 6 days postpartum and had been treated for over 3 weeks before becoming recumbent and euthanasia was performed.

Gross Pathology: The uterus was dilated and had ecchymotic hemorrhages on the serosa. The uterine contents consisted of copious fetid, mucopurulent, dark brown fluid. The mucosa was multifocally ulcerated and a caseous material was adhered

to some of the surface including caruncles. The liver was pale and fatty. Colonic contents were watery and dark.

Laboratory Results: Uterine culture: Arcanobacterium pyogenes
FA on uterus: positive for BHV-4
Viral isolation: pure isolate of BHV-4
PCR: positive using BHV-4 specific primers

Contributor's Morphologic Diagnosis: Metritis, suppurative, ulcerative, severe, diffuse with rare intranuclear inclusion bodies.

Contributor's Comment: This case is quite typical of a newly recognized syndrome in Southern U.S. dairy cattle causing refractory endometritis that is associated with Bovine Herpesvirus-4 infection. Although BHV-4 (DN-599) has been present in the United States for many years, until now it has generally been considered to be of minimal pathogenicity, or only a component of bovine respiratory disease complex (shipping fever). We have now positively identified the virus in over 35 cases of refractory metritis in cows from several dairies and all have similar histories and clinical presentations. The virus appears to be expressed only during the period of a few weeks postpartum (regardless of when infection occurred) and is associated with severe bacterial endometritis that is refractory to therapy. Pyometra is the eventual sequela. Cows often have lipidosis and/or hypocalcemia, and these metabolic changes may be cofactors in disease expression since previous investigations have suggested a link between lipidosis/milk fever and postpartum metritis. *Arcanobacterium (Actinomyces) pyogenes* was isolated from the uterus in this case and in virtually all cases either *A. pyogenes* or *E. coli* have been the pathogen(s) isolated. Like other gammaherpesviruses, BHV-4 can remain latent and animals may be infected for life. The virus can be shed in both milk and semen, as well as uterine fluid. RFLP and sequencing analysis of viral isolates indicates the current strain of BHV-4 virus in these cases is distinct from DN-599, and may be more similar (but not identical) to the European isolates of Movar 33. Very similar syndromes of BHV-4 endometritis have been reported from Europe since the early 1980's and particularly in Belgium. Fluorescent antibody assays and viral isolation are helpful, and heminested PCR using BHV-4 specific primers is definitive.

Recently, a large number of cats from a Michigan shelter were found to be seropositive and actively viremic for BHV-4, so the syndrome may not be limited to Georgia. Characteristic viral inclusion bodies in the uterine lining epithelium aid in histologic diagnosis, but they are inconsistently present (particularly if ulceration is diffuse). BHV-4 associated endometritis should be suspected in any dairy herd with a high percentage of postpartum endometritis particularly if therapy appears unsuccessful.

AFIP Diagnosis: Uterus: Endometritis, ulcerative, lymphoplasmacytic and neutrophilic, diffuse, moderate, with intranuclear inclusion bodies and intraluminal bacteria, Holstein, bovine.

Conference Comment: The contributor has provided a concise review of this emerging condition.

Contributor: University of Georgia, Veterinary Diagnostic & Investigational Laboratory, Tifton, GA, 31794

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CASE III – 2000483 (AFIP 2787410)

Signalment: 9-month-gestational age, female, Haflinger, equine

History: The fetal tissues originate from a second parity 4-year-old Haflinger mare. The mare was presented to the clinic because of premature mammary gland development. Ultrasound examination of the uterus suggested abnormal placentation. The mare was subsequently treated with antibiotics, flunixin and altrenogest. Two days later the mare aborted a breathing filly, but the foal died within minutes of expulsion. The placenta was released shortly afterwards.

Gross Pathology: The fully-haired 20.5 kg fetus was stained with meconium. Meconium was also within the oral cavity and stomach. The lungs were diffusely yellow and inflated. There was moderately severe pleural and interlobular edema. The fetal membranes were intact and 5.6 kg (27% BW); the umbilical cord was 25 cm long. The chorioallantois was severely thickened. Depressions and dimples on the allantoic surface corresponded with hypovillation on the chorionic surface.

Laboratory Results: No infectious agents were identified upon aerobic culture of lung and stomach fluid, virus culture of a tissue pool, and fluorescent antibody tests for EHV and Leptospira.

Contributor's Morphologic Diagnoses: 1. Lung: Aeration and pneumocyte immaturity, diffuse.
2. Lung: Bronchopneumonia, severe, subacute, diffuse with intraalveolar meconium (meconium aspiration)
3. Fetal membranes, allantois: Granulation tissue and gland formation, severe, diffuse with acute inflammation.

Contributor's Comment: This case is an example of uterine body pregnancy. Uterine body pregnancy is rare and poorly documented in the veterinary literature. In this condition the fetal membranes are thickened, wrinkled and have underdeveloped uterine horn segments. Abortion is presumably due to nutritional insufficiency. The nonspecific, and relatively common finding of adenomatous hyperplasia of the allantois has been associated with body pregnancies. Fetal hypoxia causes anal sphincter relaxation and the release of meconium, inducing so-called "fetal diarrhea". Meconium released before rupture of the amnion is "inhaled" and/or ingested. This case is an extreme example of meconium aspiration, and the fetal pneumonia is attributed to it.

AFIP Diagnoses: 1. Chorioallantois: Edema and fibroplasia, diffuse, moderate, with cystic glandular structures (adenomatous hyperplasia) and mild multifocal lymphoplasmacytic placentitis, Haflinger, equine.
2. Lung: Pneumonia, histiocytic, subacute, diffuse, severe, with intrahistiocytic yellow pigment (meconium) and multinucleate giant cells.

Conference Comment: Although the histologic findings in the chorioallantois are non-specific, they support an ongoing chronic process. Features of chronicity include diffuse fibrosis, adenomatous hyperplasia, and severe thickening of the placenta; a typical placenta is approximately 11% of the fetal body weight. Adenomatous hyperplasia, with or without cyst formation, can be focused within the epithelium or form glandular masses in the allantoic stroma. One study reported that 61 of 63 cases of adenomatous hyperplasia were associated with other placental and fetal conditions (e.g. chronic or chronic-active placentitis, placental edema, and fetal diarrhea) suggesting this lesion is secondary to chronic irritation.

Contributor: University of Pennsylvania, School of Veterinary Medicine, New Bolton Center, Kennett Square, PA 19348

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CASE IV – 2441495 (AFIP 2788619)

Signalment: 8-year-old, female, Chihuahua, canine

History: This dog was reported to have a lymphadenopathy for 3 weeks. At the time of ovariohysterectomy, a tumor was noted on the right ovary.

Gross Pathology: The ovarian mass was not described grossly.

Laboratory Results: None.

Contributor's Morphologic Diagnosis: Right ovary: most consistent with a dysgerminoma.

Contributor's Comment: The ovary had occasional primary and secondary follicles interspersed in a diffuse population of germ cells that totally effaced the ovarian architecture. The tumor cells were in diffuse sheets of cells or somewhat individualized in regions with hyperchromatic nuclei with solitary nucleoli and abundant cytoplasm. Clusters of small lymphocytes were also present randomly in the tissue. Mitotic activity ranged up to 5/HPF with occasional bizarre mitoses. The histologic features were most consistent with a dysgerminoma, the ovarian counterpart of the testicular seminoma.

Dysgerminomas are exceptionally rare ovarian tumors in dogs. A search of the AHDL database found only nine diagnoses in dogs from January 1983 to May 2001 in comparison to a total of 42 granulosa cell tumors over the same time frame in dogs. Little work has been performed in the immunohistochemistry of ovarian tumors in the dog, but multiple procedures have been used in man. Studies in our lab have indicated that seminomas of the testicle stain consistently with p53 (paper in progress). Dysgerminomas may metastasize in 10-20% of the cases and are indicated to be radiation sensitive.

AFIP Diagnosis: Ovary: Dysgerminoma, Chihuahua, canine.

Conference Comment: Histologically indistinguishable from testicular seminomas, dysgerminomas develop from germ cells prior to differentiation. Dysgerminomas have been reported in all domestic species, are malignant, and can metastasize. Other tumors arising from germ cells include teratomas and rarely reported

embryonal carcinomas. Teratomas arising from germ cells undergo somatic differentiation, producing endodermal, mesodermal, and ectodermal tissue.

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