Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2020-2021

Conference 2

26 August 2020

Joint Pathology Center
Silver Spring, Maryland

CASE 1: S17/4906 (4116732-00)

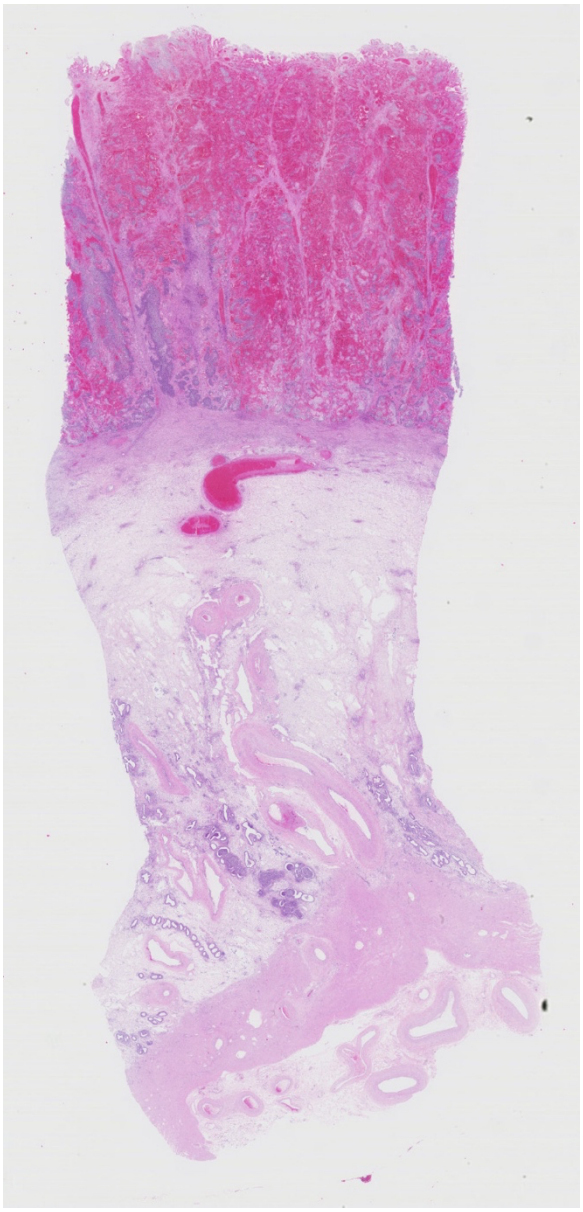
Signalment: 4.75Y, female, Montbéliarde, *Bos taurus*, bovine

History: On a dairy farm in the canton of Jura in northwestern Switzerland, four cows presented with a history of persistent fever over several days. Two to three weeks prior, another cow from the same farm suddenly died without noticeable premortal symptoms. A field necropsy conducted on this cow revealed nodular hemorrhagic lesions in the lungs, which tested positive for *Bacillus anthracis*. The four cows with fever repeatedly tested negative for *B. anthracis* via blood culture. Three of these four cows spontaneously recovered; the fourth cow showed signs of an incipient abortion and was treated once with penicillin. The next day, the veterinary authorities decided to submit the cow for necropsy to elucidate the cause of this nonspecific feverish illness. Sixteen hours after treatment, the cow was transported alive to the pathology laboratory for diagnostic testing, euthanasia, and necropsy under special biosafety measures.

Gross Pathology: A small amount of red-tinged, viscous vaginal discharge was present. Further examination demonstrated marked subcutaneous edema of the ventral abdomen, in addition to significant enlargement of the iliac lymph nodes. Examination of the uterus yielded an edematous, expired fetus in blood-tinged amniotic fluid. The placentomes were moderately hemorrhagic and friable and the placenta was easily detachable. The remainder of the necropsy, including the spleen, was grossly unremarkable.

Laboratory results: Giemsa-stained smears and bacterial cultures from blood, spleen, liver and kidney were negative, but antibiotic residues were identified. *B. anthracis* was isolated culturally in large and small amounts from placenta and iliac lymph node, respectively. The presence of *B. anthracis* in placental tissue and iliac lymph node was confirmed with a gamma-phage lysis assay and PCR specific for *B. anthracis*.

Microscopic description: Uterus, placentome:
The placentomal tissue shows diffuse and extensive acute hemorrhage and coagulative necrosis affecting both fetal cotyledonary and maternal caruncular parts. Remaining epithelial cells are commonly swollen and pale (hydropic degeneration). The tissue is multifocally infiltrated by moderate to large numbers of mostly degenerated neutrophils. Within the necrosis and hemorrhage, there are numerous long, pale basophilic bacilli evident, often arranged in heaps. The transition zone to the endometrium is heavily infiltrated by degenerated neutrophils. Vessels in this area as well as in the subjacent endometrium exhibit



Placentome and uterus, ox. There is diffuse severe hemorrhage of the placentome (top) with severe edema of the transitional zone and endometrium (bottom). (HE, 5X)

fibrinoid necrosis and neutrophilic infiltration of their wall, associated with perivascular fibrin leakage. The endometrium presents with a massive edema in the lamina propria. Lymph vessels are severely dilated, show loss of endothelial cells, and contain few degenerated neutrophils within their lumina. The endometrial interstitium and the endometrial glands are multifocally infiltrated by numerous mostly degenerated neutrophils.

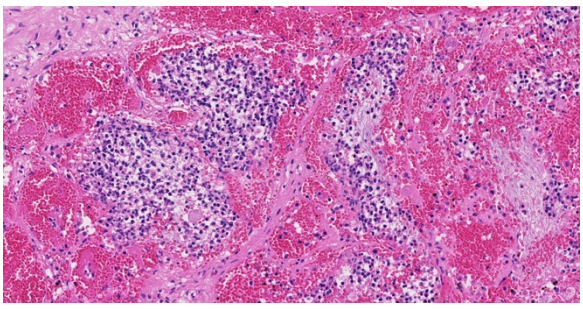
Gram stain (not submitted): The bacilli are gram-positive.

Iliac lymph node (not submitted): The lymphatic tissue and the adjacent perinodal fatty tissue shows extensive necrosis and infiltration with degenerated neutrophils, associated with extensive edema and fibrinoid vasculitis. In contrast to the placentome tissue, only few gram-positive rod-shaped bacteria are evident.

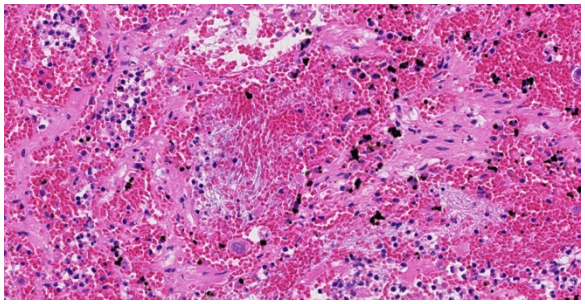
Liver (not submitted): Multifocal randomly distributed small foci of necrosis and neutrophilic infiltration are evident, consistent with an embolic hepatitis.

Contributor's morphologic diagnosis:
Uterus: Endometritis and placentitis, hemorrhagic, necrotizing and suppurative, severe, diffuse, acute, with fibrinoid vasculitis, severe edema and numerous bacilli.

Contributor's comment: The postmortem and histological findings in combination with the laboratory results are compatible with a localized, non-septicemic form of anthrax. This worldwide occurring zoonotic and usually fatal disease is caused by the Gram-positive, large rod-shaped, spore-forming bacterium *Bacillus anthracis*.[2,4-5,9-10] The vegetative form of *B. anthracis* is almost exclusively found in vital tissues of warm-blooded animals because of its need for a low oxygen pressure.[11] Outside a host, *B. anthracis* forms non-proliferating endospores, which can persist in the environment for decades and are



Placentome, ox. There is diffuse congestion and hemorrhage of the placentome with necrosis of epithelium and trophoblasts and infiltration of numerous viable and necrotic neutrophils primarily within fetal cotyledonary tissue. There is extensive polymerization of fibrin within the interstitium of caruncular tissue. (HE, 257X)



Placentome, ox. Within areas of hemorrhage and necrosis, haphazard arrays of numerous filamentous bacilli are present. (HE, 327X)

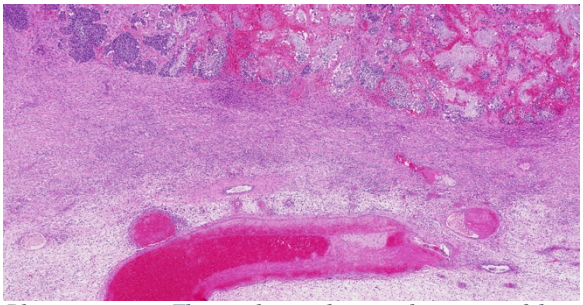
resilient to ultraviolet radiation, dehydration, and temperature extremes, in addition to many methods of disinfection, save for oxidizing agents.[2,10] Nevertheless, in oscillating periods of precipitation, endospores may germinate spontaneously into vegetative forms and may proliferate in the environment to a limited extent.[10] According to the Centers for Disease Control and Prevention, *B. anthracis* endospores are commonly found in the soil of agricultural regions of Central and South America, sub-Saharan Africa, central and southwestern Asia, southern and eastern Europe, and the Caribbean.[2] Ruminants are highly susceptible to the disease and are usually infected upon grazing endospore-laden pastures. The ingestion of endospores typically causes a peracute, massive bacteremia only a few hours before death.[10-11] In more resistant species, such as horses, swine, and carnivores, the disease is decelerated and the infection is usually localized to the area of endospore entry, which is most likely the gastrointestinal tract [11]. Less frequently, the localized form may also be seen in ruminants, manifesting as enteric or pulmonary anthrax.[11] In non-septicemic anthrax, death is usually a sequela to the extensive tissue damage caused by bacterial exotoxins.[10-11]

In humans, most common form is cutaneous anthrax acquired by endospore-contaminated wounds.[11] Occasional cases of the alimentary form occur after consumption of contaminated and poorly cooked meat. In earlier times, the third form, pulmonary anthrax, was a frequent human disease especially affecting workers of lane-processing and tanning industries, where bacterial endospores easily aerosolized from contaminated skins and wool.[10-11]

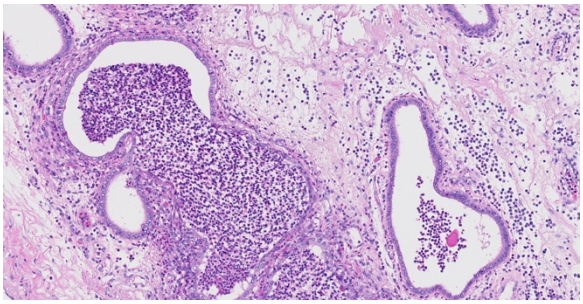
The mechanism by which *B. anthracis* invade tissues and lymph vessels is poorly understood, but abrasions or wounds in skin and mucosae as a prerequisite have been proposed. [2,4-5,9-10] In the enteric form, spores and/or vegetative forms are able to evade gastric acidity and reach the intestine by means of peristalsis.[10] Germination of endospores likely occurs either in the mucus layer of the mucosa or within Peyer's patches after phagocytosis by M cells.[4-5,10]

The two principal virulence factors of *B. anthracis* are the polypeptide capsule to evade phagocytosis and the toxin complex causing cell lysis. The latter being an A-B-class toxin consists of the three proteins protective antigen (PA), edema factor (EF), and lethal factor (LF).[9-11] PA binds to receptors on the host cell surface, where it is activated by cleaving. Heptamers of activated PA form pores with competitive binding sites for either EF or LF. PA-EF (edema toxin) or PA-LF (lethal toxin) complexes are internalized by endocytosis, followed by release of EF and LF into the cytoplasm.[6] EF is an adenylate cyclase causing edema by disturbance of water and ion exchange. LF is a protease causing cell death by interruption of the MAP kinase pathway.[4-5,10-11]

The typical gross findings of ruminants succumbed to septicemic anthrax comprise rapid autolysis, hemorrhagic discharge from orifices, and an enlarged, dark red, friable spleen.[5,10] Histologically, inflammation is usually absent, as animals typically succumb quickly within hours of development of acute bacteremia.[5,10] In



Placentome, ox. There is hemorrhage and necrosis of the placentome at top, and, infiltration of large number of neutrophils in the transitional zone (middle), and thrombosis of endometrial veins (bottom). (HE, 40X)



Endometrium, ox. The endometrium is diffusely edematous and endometrial glands contain numerous viable and necrotic neutrophils (HE, 155X)

alimentary anthrax, gross lesions include segmental to diffuse hemorrhagic enteritis with transmural edema and hemorrhage. Mesenteric lymph nodes may also be enlarged, edematous, and hemorrhagic.[5,9] Pulmonary anthrax is characterized by hemorrhagic consolidation of portions of the lungs, associated with interstitial and mediastinal edema and mediastinal hemorrhagic lymphadenitis.[9] Infected carnivores, horses and swine most commonly present with extensive edema, hemorrhage and swelling in the oropharyngeal region and the neck, or suffer from the enteric form.[9] In histology, localized infections exhibit extensive necrosis, edema and hemorrhage associated with fibrinoid vasculitis and the presence of variable numbers of bacilli.[9]

Abortion as a sequela to anthrax infection may occur as in other illnesses with fever. The presence of *B. anthracis* in bovine abortion material, however, has been described only once.[3] In the present case, an initial nonlethal bacteremia that resolved spontaneously is suspected to have spread to the placenta, which consequently harbored a local bacterial proliferation and ultimately termination of the fetus. Here, the concurrent necrotizing iliac lymphadenitis is consistent with the spread of bacteria to the regional lymph nodes.

The conduction of necropsies on animals with a suspicion of anthrax should be avoided unless special biosafety infrastructure is available to prevent personnel and environmental contamination. In suspicious cases, blood and all sorts of discharge from body orifices should be tested with sensitive methods, such as culture and PCR, for the presence of *B. anthracis* before opening the carcass, bearing in mind that they may be false negative due to lack of septicemia or antibiotic treatment.[1,7] Thus, it is important to still consider anthrax as a differential in cases of necrohemorrhagic enteritis, pharyngitis, lymphadenitis, or red-tinged discharge from body orifices despite negative blood cultures.

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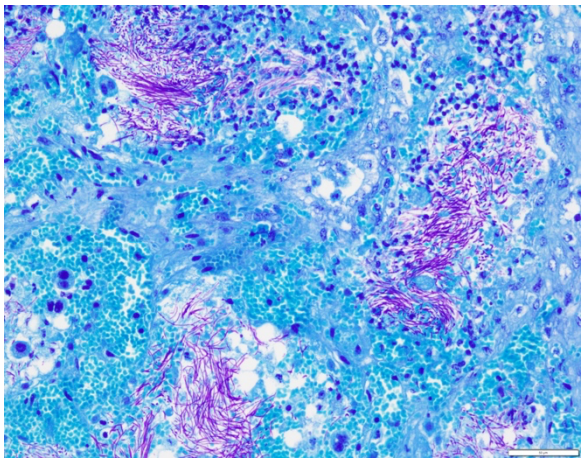
JPC diagnosis:
Uterus and placenta: Placentitis, necro-hemorrhagic and suppurative, diffuse, severe, with placental vasculitis, edema, mild suppurative endometritis, and numerous extracellular bacilli, Montbéliarde, bovine.

JPC comment: The contributor provided a concise and comprehensive review of the clinical and pathologic features of this entity. In addition, *B. anthracis* is an illustrious member of the Select Agents and Toxins List under the U.S. Federal Select Agent Program (7CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73), administered jointly by the U.S. Department of Health and Human Services, the U.S. Department of Agriculture, and the Center for Disease Control



Placentome, ox. A Giemsa stain demonstrates sheaves of bacilli scattered throughout the necrotic debris. (Giemsa, 200X)

and Prevention. In fact, *B. anthracis* is categorized as a Tier 1 agent, along with Ebola virus, smallpox virus, *Yersinia pestis*, and others. This categorization necessitates additional considerations with respect to research circumstances (facility registration, control, disposal, etc), but diagnostic labs are largely exempt from these requirements. If a specimen is presented, and is diagnosed with a select agent, the facility is expected to: 1) officially transfer the specimen, or destroy the specimen on-site by a recognized sterilization or inactivation process within 7 days, 2) until transfer or destruction, the agent or toxin is secured against theft, loss, or release, 3) original specimens containing the select agent or toxin are transferred or destroyed within 7 days, and 4) the identification of the select agent or toxin is reported to USDA APHIS or CDC.

The main FDA approved vaccine (Biothrax), is made from cell-free filtrates of microaerophilic cultures of an avirulent, nonencapsulated strain of *B. anthracis*. CDC currently recommends vaccination of certain laboratory workers who work with *Bacillus anthracis*, people who handle potentially infected animals or their carcasses, some military personnel, and some emergency and other responders whose response may lead to exposure. Additional research continues, and Protective Antigen domain 4 and the combination of nanoparticles and bacteriophage T4 have been recent targets for vaccine development.[8,12]

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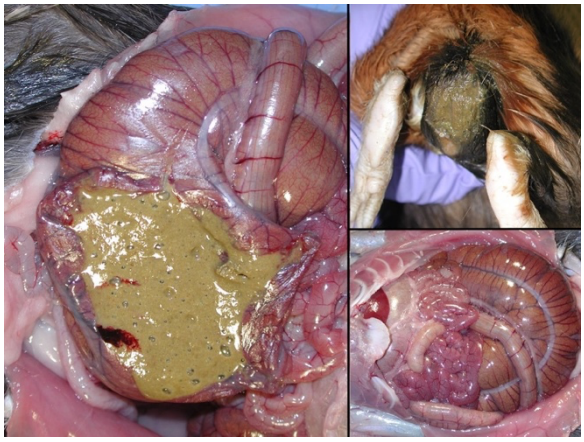
CASE 2: 17-0036 (4101204-00)

Signalment: 2-month-old male guinea pig (*Cavia porcellus*).

History: This animal had been received from a commercial supplier several weeks previously and was experimentally unmanipulated. It had been clinically normal, though generally nervous and skittish until that morning, when it was found recumbent, semi-moribund and in severe



Cecum, colon: The perineum is soiled, and the cecum and large intestine are filled with green soft feces. (Photo courtesy of the Division of Laboratory Animal Resources, University of Pittsburgh, www.dlar.pitt.edu)

distress, with possible pain when the abdomen was palpated. It expired spontaneously shortly before the planned sacrifice and necropsy.

Gross Pathology: The perineum was soiled, and cecum and large intestines were moderately distended with greenish soft to liquid feces.

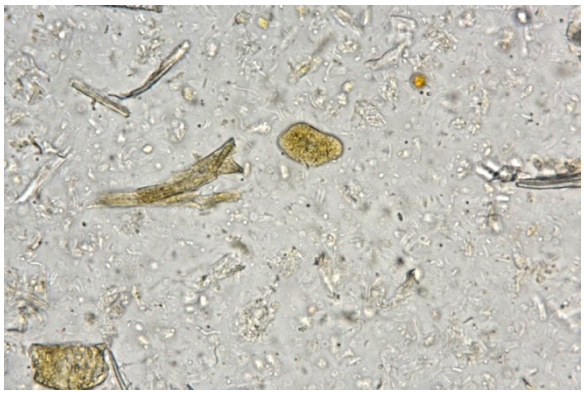
Laboratory results: Fecal culture for enteric pathogens was negative. Fecal examination for *Cryptosporidium* and *Eimeria* were negative. PCR for *C. difficile* toxin was negative.

Microscopic description: Full thickness pieces of cecum and colon are examined. There is a patchy to near diffuse (depending on section) acute necrotizing and ulcerative process present, involving primarily superficial mucosal regions, including enterocyte degeneration & exfoliation along with a florid granulocytic infiltrate. Inflammation extends into deeper mucosal regions in many areas as well. Admixed with superficial epithelial and luminal necrotic debris, but also seen extending into deeper mucosal regions are numerous protozoan organisms. These are consistent morphologically with both cyst and trophozoites forms of Entamoeba. Erythrophagocytosis is frequently present within trophozoites structures (abundance of red cell ingestion not evident in all sections).

Contributor's morphologic diagnosis:
Typhlocolitis, necrotizing and ulcerative, acute, patchy to diffuse, marked, with numerous surfaces and more deeply invasive protozoal organisms consistent with *Entamoeba* sp. (Observed both in tissue section and on wet mount fecal smears).

Contributor's comment: *Entamoeba* infection and disease (amoebiasis) in mammalian species primarily affects humans and other primates and is generally associated with *Entamoeba histolytica*. Other mammals such as cats and dogs can occasionally be spontaneously infected with this organism although clinical disease is rare. Amoebiasis in reptiles, typically associated with *Entamoeba invadens* is well recognized, being commonly reported in snakes and lizards. In search of animal models of disease, rats, mice, guinea pigs, and rabbits have been experimentally infected, typically by injecting large numbers of trophozoites directly into the lower bowel per annuum, or surgically introducing them into the cecum.[5] However, rodents appear to be naturally resistant to infection, the cellular and molecular basis of such immunity not being completely understood.[4]

Innate *Entamoeba* species have been identified in a variety of rodents and are generally considered to be commensal inhabitants of the cecum; *Entamoeba muris* is well described in rats, mice and golden hamsters.[2]

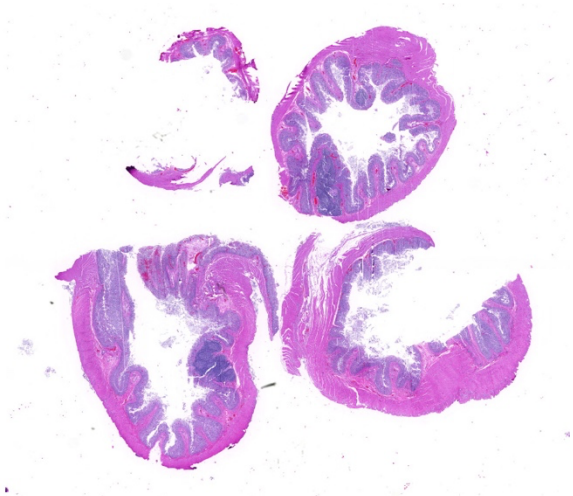


Fecal wet mount: Trophozoites of Entamoeba sp. identifiable by a ring of coarse granules inside the cell membrane. (Photo courtesy of the Division of Laboratory Animal Resources, University of Pittsburgh, www.dlar.pitt.edu)

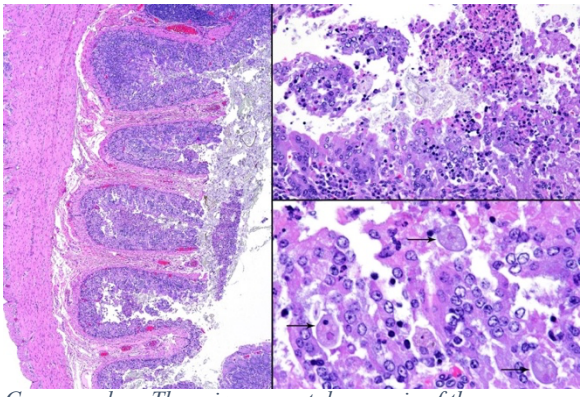
Entamoeba cobayae was described in guinea pigs by Walker in 1908, although this is now considered synonymous with the more commonly designated *E. caviae*.[3] Indeed *E. caviae* is known to be a common inhabitant of the laboratory guinea pig and is widely considered to be non-pathogenic.[6]

In earlier days, *Entamoeba* were identified and classified on the microscopic basis of nuclear structure of trophozoites and cysts found in stool preparations.[3] The basis for such identifications depended greatly on the skill and expertise of the microscopist and previously, no means were available to confirm results other than reexamination of the sample by a more experienced microscopist. Furthermore, morphologically identical species or genetic variants could not be distinguished solely on this basis.[14] Apart from size, number and appearance of the nuclei, chromatoid bar appearance and several other features, there are few criteria to differentiate between organisms and the use of minor morphological differences to separate species may turn out to be not reliable.[7] A case in point involves *Entamoeba dispar*; a separate but non-invasive and non-pathogenic Entamoeba species in humans which is microscopically indistinguishable from *E. histolytica* and does not require treatment.[7,13]

Examination of fecal wet mounts in this case revealed abundant trophozoites structures



Cecum, colon: Multiple sections of cecum and colon are submitted for examination. (HE, 7X)

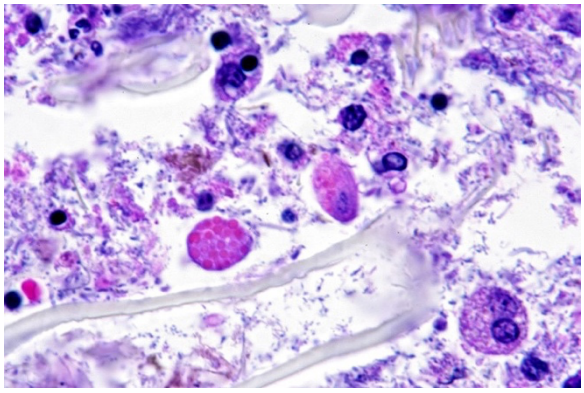


Cecum, colon: There is segmental necrosis of the superficial mucosa. (left). There is a fibrinonecrotic membrane overlying areas of necrosis (top right) and within areas of necrosis and in the overlying fibrino-necrotic membrane there are numerous trophozoites of Entamoeba histolytica. (HE, 40-400X) (Photo courtesy of the Division of Laboratory Animal Resources, University of Pittsburgh, www.dlar.pitt.edu)

compatible with Entamoeba generally measuring between ~20 – 26 µm in diameter, with nuclei ~5.0 – 6.2 µm possessing a ring of coarse peripheral granules inside the nuclear membrane. Cysts were present, though rare, measuring ~20 µm in diameter. Trichrome staining is pending to try and identify the number of nuclei present, as this can be an important consideration in distinguishing between pathogenic and non-pathogenic types of the organism.[2]

Alternative means of identification beyond morphology include isoenzymatic methods, immunological analysis (both antibody and antigen detection) and molecular diagnostics (both conventional and Real-time PCR).[9] Certainly the majority of molecular diagnostic tests available focus on distinguishing *E. histolytica, E. dispar* and other non-pathogenic species known in humans.

Initial PCR fecal testing on this animal was negative for *E. histolytica, E. dispar and E. moshkovskii),* ruling these organisms out as etiologic considerations (though they were not thought to be likely agents based on historical knowledge of their host range). Additional frozen samples from the case were submitted to a laboratory for DNA sequencing. Results indicated that the organism appears to be an uncharacterized species of *Entamoeba* that is



Cecum, colon: Numerous trophozoites of Entamoeba sp. contain fragments of ingested erythrocytes. (HE, 400X) (Photo courtesy of the Division of Laboratory Animal Resources, University of Pittsburgh, www.dlar.pitt.edu)

most closely related to *Entamoeba coli*, with ~97% homology. Unfortunately, *Entamoeba caviae* does not have sequence data available in GenBank and therefore could not be completely excluded based on known genomic standards.

The possibility that the primary necrotizing enteric disease present in this case was of another etiology and that the amoebic organisms present had proliferated in response to this was considered, but thought less likely due to 1) the presence of deep invasion of organisms into the mucosa, 2) the negative culture and PCR results for other known causes of typhlocolitis in Guinea Pigs and 3) the lack of morphological evidence of other etiologic agents such *C. piliforme* via special stains. Another indirect consideration suggesting a primary *Entamoeba* pathogenesis in this case is the high degree of erythrophagocytosis noted in section. Phagocytic activity by pathogenic Entamoeba trophozoites (including *E. histolytica*) has been accepted as a qualitative pathogenicity factor and one associated with virulence mechanisms responsible for invasive capacity.[10]

In summary, despite the lack of substantial similar reports in the literature, this case of necrotizing typhlocolitis was considered likely of a primary *Entamoeba* etiology, although other infectious cofactors could not be completely excluded. The exact species of Entamoeba involved was undetermined, as was the reason for the susceptibility of this animal to infection.

Contributing Institution:
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JPC diagnosis:
Colon: Colitis, necrotizing, multifocal, moderate, with moderate numbers of extracellular amoebic trophozoites, guinea pig, rodent.

JPC comment:
The contributor highlights important background information for this entity, as well as obstacles sometimes encountered in veterinary medicine. As computational pathology matures as a field, we expect diagnostic capabilities to increase dramatically. Perhaps sequences for *E. caviae* will be available in the near future.

In humans and non-human primates (NHP), the primary sites of dissemination are the liver (via the portal circulation), and less commonly, the lung and brain. Fatal amebiasis with abscess formation has been reported in multiple primate species.[8]

The pathogenesis of tissue damage caused by amebae species are:

1. Adhesion to mucus by lectins
2. Enzymatic breakdown of protective mucus
3. Lectin-mediated adherence to host epithelium

E. histolytica releases cysteine proteases that cause damage to mucosal epithelium and attract inflammatory cells, both of which lead to characteristic ulcerative colitis with flask-shaped ulcers. Microscopically, amebae are surrounded by a clear halo with extensive pseudopodia and possess a nucleus with a dark karyosome and peripheral chromatin clumps. The cytoplasm appears foamy and they frequently phagocytize erythrocytes, which makes them difficult to distinguish from activated macrophages. Their cytoplasm often contains glycogen, making amebae PAS positive.[13]

Amoebae continue to be important zoonotic pathogens of concern, and have been found in

Table 1. Correspondence between Historic, Binomial, and Sequence-based Nomenclature for *Entamoeba* Species in Primates

Dobell nomenclature[a]	Current species names	Identified in primates (incl. humans)	Molecular identification in NHPs
E. histolytica	*E. histolytica*	*E. histolytica*	Y[b]
	E. dispar	*E. dispar*	Y
	E. hartmanni	*E. hartmanni*	Y
	E. nuttalli	*E. nuttalli*	Y
	E. moshkovskii	*E. moshkovskii* (complex)	N
	E. polecki	*E. polecki* ST1[c]	N
		E. polecki ST4	Y
	E. chattoni	*E. polecki* ST2	Y
	E. struthionis	*E. polecki* ST3	N
	E. bangladeshi	*E. bangladeshi*	N
	E. suis	*E. suis*	Y[b]
E. coli	*E. coli*	*E. coli* ST1	Y[b]
		E. coli ST2	Y
E. gingivalis	*E. gingivalis*	*E. gingivalis* ribodeme 1[d]	N
		E. gingivalis ribodeme 2	N
	None	*Entamoeba* RL3[e]	Y
		Entamoeba RL6	Y
		Entamoeba RL7	Y
		Entamoeba CL8[f]	Y

[a]Dobell's nomenclature is that proposed in his 1919 monograph [3].
[b]Identified in captive NHPs only, to date.
[c]Subtypes (ST) are distinct small-subunit ribosomal DNA sequence variants that clearly fall within a named species.
[d]Ribodemes are small-subunit ribosomal DNA variants detected by restriction enzymes.
[e]Ribosomal (RL) [14] lineages indicate complete mall-subunit ribosomal DNA sequences that are clearly distinct from all named species.
[f]Conditional (CL) [34] lineages indicate partial small-subunit ribosomal DNA sequences that are clearly distinct from all named species.

wild and captive prosimians, apes (*Balamuthia mandrillaris, E. histolytica, E. dispar, Dientamoeba fragilis, and other members of Entamoeba spp, Dientamoeba spp, and Iodoamoeba sp.*), reptiles (*E. invadens*), amphibians (*E. ranarum*), and domestic pigs (*D. fragilis*). Interestingly, given its aquatic environment, captive tapir in South America have developed necrosuppurative meningo-encephalitis from infection by *Naegleria fowleri*.[12] This amoeba is often known as the "brain-eating amoeba" and infects humans by extension from nasal passages and through the cribriform plate, causing primary amebic meningoencephalitis (PAM). Death is acute, and little inflammation develops.[15]

Recent molecular investigations have elucidated the variety of *Entamoeba* species that infect humans and nonhuman primates. Leveraging current techniques using monoclonal antibodies, DNA hybridization, SSU-rDNA restriction fragment length polymorphism, and DNA sequencing, a distinct species infecting nonhuman primates was isolated from previously presumed *E. histolytica* cases and named *Entamoeba nuttalli*. In total, there are six named *Entamoeba* species that infect nonhuman primates (*E. coli*, *E. polecki*, *E. histolytica*, *E. nuttalli*, *E. dispar*, and *E. hartmanni*), as well as four that are not yet named, but identified by unique gene sequences (*Entamoeba* RL3, RL6, RL7, and CL8).[1,11]

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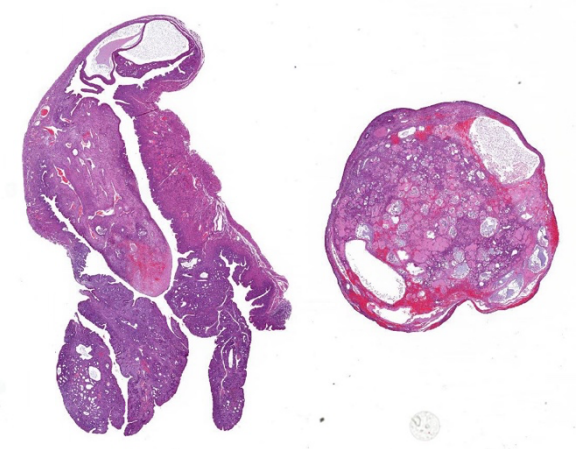
CASE 3: 15-2890 (1-4) (4084553-00)

Signalment: Twelve-month-old, intact female NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ (NSG) mouse, *Mus musculus*.

History: The animal was part of a cohort of NSG retired breeders experimentally infested with *Syphacia obvelata*. Health records indicated that the animal was clinically normal for 4-6 months post-infection. Subsequently, decreased appetite and marked weight loss were noted, along with lethargy and dehydration. The animal was euthanized and submitted for necropsy.

Gross Pathology: The uterine horns were diffusely thickened and slightly dilated and measured 5 to 6 mm in diameter. Upon opening, the uterine lumen was partially filled with a moderate amount of clear yellow viscous material.



Uterus, mouse. Sections of uterus are presented for examination. The uterus (right) is largely effaced by a multicystic neoplasm, and a large mural polyp expands the uterine lumen (left). (HE, 5X)

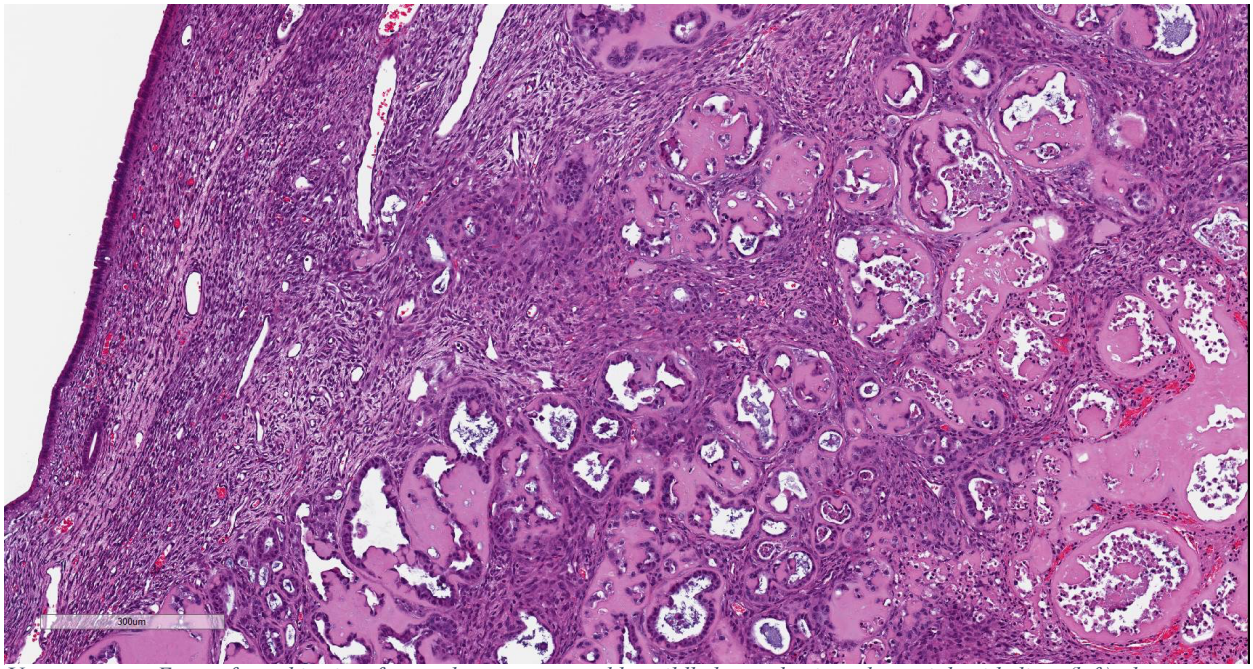
Laboratory results: A swab of the uterine mucoid content was submitted for aerobic and anaerobic cultures, and no bacteria were isolated upon microbiology.

Microscopic description:
Uterus. Multifocally infiltrating the endometrium and subjacent myometrium and filling the lumen, there is a poorly demarcated, unencapsulated, scarcely cellular epithelial neoplasm composed of acini lined by a simple columnar epithelium, nests and ribbons. The cells are associated with an abundant extracellular eosinophilic hyaline material. Cells are cuboidal and polygonal, with distinct borders and a moderate amount of amphophilic and occasionally vacuolated cytoplasm. Nuclei are round to oval, with finely granular to vesicular chromatin and one or multiple prominent magenta nucleoli. There is moderate anisocytosis and anisokaryosis. The mitotic index is 15 (mitoses per 10 high power fields 40x). Scattered throughout the neoplasm, there are multifocal areas of coagulative necrosis and hemorrhages. In the adjacent section of more preserved uterus, there is moderate multifocal cystic dilation of endometrial glands, without increased mitotic activity of evidence of proliferative changes of the epithelium. There is also a focal endometrial polyp composed of fibrovascular stroma without glands and infiltrated by a small numbers of hemosiderin laden macrophages. The surface of the polyp is lined by a simple columnar epithelium, with focal ulceration associated with a neutrophilic and fibrinous exudate.

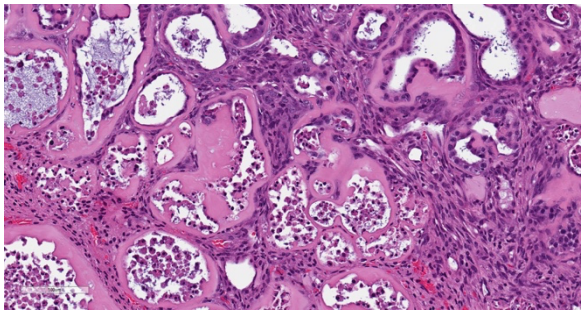
Special stains (Periodic acid–Schiff, Masson's trichrome and Congo red stains): The hyaline ground substance is negative for Congo red and positive for PAS. This material also stained blue with Masson's trichrome. Additionally, the neoplastic cells occasionally contain PAS-positive intracytoplasmic granules.

Immunohistochemical stain (laminin):
The hyaline ground substance is positive for laminin. Additionally, the neoplastic cells exhibited strong cytoplasmic positivity.

Contributor's morphologic diagnosis:
Uterus: Yolk sac carcinoma, parietal.
Uterus: Endometrial glandular cystic dilation, multifocal, moderate.
Uterus: Endometrial stromal polyp.



Uterus, mouse. Except for a thin rim of normal stroma covered by mildly hyperplastic endometrial epithelium (left), the endometrial stroma is replaced by a neoplasm composed of nests of polygonal epithelium surrounded and separated by abundant brightly eosinophilic protein matrix. Nests of neoplastic cells are separated by endometrial stroma. (HE, 84X)



Uterus, mouse. Higher magnification of nests of polygonal, often columnar neoplastic epithelial cells surrounded by their eosinophilic protein secretions. Neoplastic cells engulfed by this matrix are often necrotic. (HE, 200X)

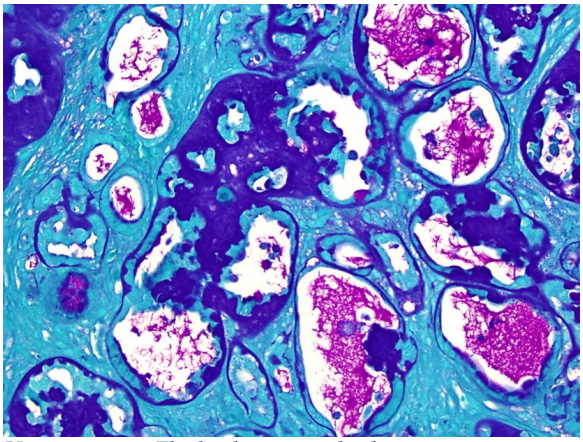
Contributor's comment: Yolk sac carcinomas, also known as malignant tumors of the endodermal sinus, represent a rare spontaneous neoplastic variant of germ cell tumors[3] and have been previously reported in experimentally manipulated or control rodents.[1,5,9,17,18] Spontaneous yolk sac carcinomas may be more commonly seen in BDII/Han rats,[3] and have been found at the level of the reproductive system both in the ovaries and the uterus. Eight cases of primary uterine yolk sac carcinomas have been described in mice.[18] Yolk sac carcinomas can be also experimentally induced in both rats and mice through implantation of an extraembryonic tissues or whole egg,[10,15] or through intraperitoneal injection of neoplastic ascitic fluid.[10] Their spontaneous occurrence in the testis has been reported in the SD rat and in a Swiss albino mouse.[2,6]

In humans, primary yolk sac tumors of the endometrium are extremely rare.[7] Only nine cases have been reported in the literature, among which seven cases are pure yolk sac tumors and only two cases are in coexistence with endometrial carcinoma.[11,12] On the contrary, they are mostly discovered in the gonadal tissues infants and adolescents (median age, 19 years), although 10% to 15% of the cases may arise in a variety of midline extragonadal sites with an axial distribution pattern.[13] Yolk sacs of rodents and humans display several morphologic differences which give rise to the peculiar appearance of those tumors in the two species.[18] Diagnostic feature of yolk sac carcinomas in humans include Schiller-Duval bodies and hyaline globules that stained positively for alpha fetoprotein.

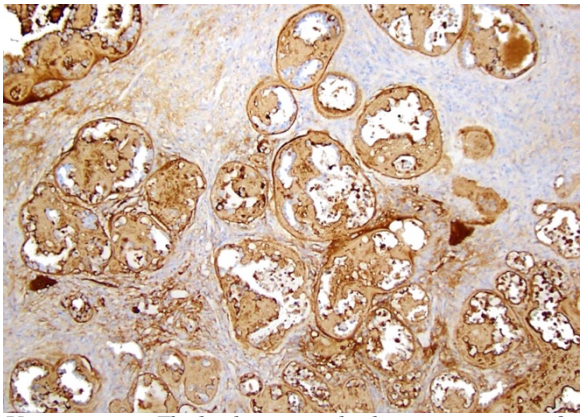
In rodents, both spontaneous and induced yolk sac tumors have histologic patterns mimicking one or two layers of fetal membranes which are represented by the parietal and the visceral yolk sac. It has been reported that inducible yolk sac carcinoma is composed of endodermal cells and contains mesenchymal, trophoblastic and mesodermal cells.[17]

Parietal yolk sac tumor cells produce an abundant, eosinophilic, PAS-positive matrix in which nests and cords of neoplastic cells are embedded. The neoplastic cells often form rosettes, cords or papillary structures, and intracytoplasmic PAS-positive granules are also present.[3,9,18] Visceral yolk sac tumor cells are large, cylindrical, lack of PAS-positive droplets and are positive for alpha fetoprotein.[3,6,14]

The hyaline ground substance characteristic of parietal yolk sac tumors is reminiscent of Reichert's membrane, a basement membrane that separates the visceral and parietal layers of the rodent yolk sac.[9] Reichert's membrane is a specialized basement membrane that is not



Uterus, mouse. The hyaline ground substance is negative for Congo red and positive for PAS. This material also stained blue with Masson's trichrome. Additionally, the neoplastic cells occasionally contained PAS-positive intracytoplasmic granules. (Masson's trichrome, 100X). (Photo courtesy of: Laboratory of Comparative Pathology, Hospital for Special Surgery, Memorial Sloan Kettering Cancer Center, The Rockefeller University, Weill Cornell Medicine, https://www.mskcc.org/research-areas/programs-centers/comparative-medicine-pathology)



Uterus, mouse. The hyaline ground substance is positive for laminin. Additionally, the neoplastic cells exhibited strong cytoplasmic positivity.(anti-laminin, 100X). (Photo courtesy of: Laboratory of Comparative Pathology, Hospital for Special Surgery, Memorial Sloan Kettering Cancer Center, The Rockefeller University, Weill Cornell Medicine, https://www.mskcc.org/research-areas/programs-centers/comparative-medicine-pathology)

present in the human placenta. It surrounds the embryo, connects trophoblast cell layer on the maternal side and the parietal endodermal cells on the embryonic side, and passively filters nutrients; therefore, proper functioning of Reichert's membrane plays a crucial role in normal embryo development. Several methods have been reported to better characterize this hyaline substance, including silver impregnation, mucicarmine, PAS, PTAH, and staining for collagen.[17] Both the neoplastic cells and the matrix stain positive for laminin,[3,14,18] as documented here.

Presenting clinical signs in affected rodents include abdominal distension, ascites, and intraabdominal palpable masses. Metastatic spread of yolk sac carcinomas has been reported both in mice and rats, with neoplastic foci detected at the level of the peritoneal cavity, mediastinum and lungs.[8,18] In the present case, no metastatic spread was evidenced in any of the organ examined.

The pathogenesis of these tumors remains poorly understood, as yolk sac tumors have been reported both in virgin animals,[8,18] and in animals that have been mated.[10] In the present case, the mouse was a retired breeder with a long term history of pregnancies and parturitions; however, the animal has not been mated since 6 months prior.

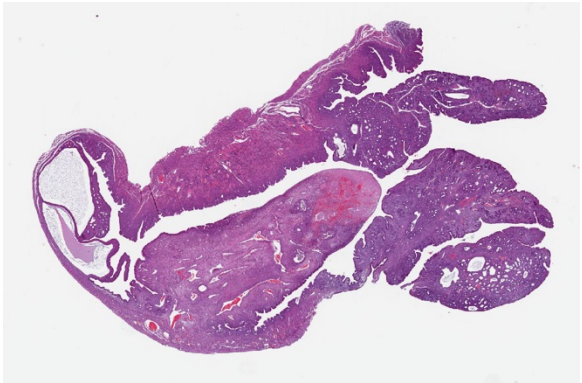
Contributing Institution:
Laboratory of Comparative Pathology
Hospital for Special Surgery
Memorial Sloan Kettering Cancer Center
The Rockefeller University, Weill Cornell Medicine

https://www.mskcc.org/research-areas/programs-centers/comparative-medicine-pathology

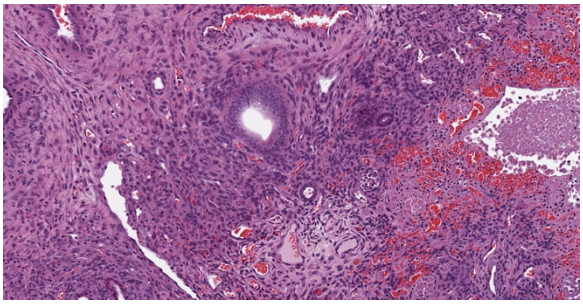
JPC diagnosis:

1. Uterus: Yolk sac carcinoma, mouse, rodent.
2. Uterus: Endometrial stromal polyp.
3. Uterus, endometrium: Cystic endometrial hyperplasia, diffuse, mild.

JPC comment: The contributor succinctly summarized the current knowledge about yolk sac carcinomas. This is a rare diagnosis to make, and much more commonly diagnosed in young human boys.



Uterus, mouse. A large elliptical polyp projects into the lumen. The tip of the polyp is necrotic and hemorrhagic. There is diffuse mild cystic endometrial hyperplasia. (HE, 10X)



Uterus, mouse. The polyp is composed primarily of endometrial stroma with a few entrapped glands. (HE, 200X)

While this is a rare entity, it presents a good opportunity to review embryology and the development of the reproductive system. Unlike non-mammalian species, the mammalian embryo does not rely on stored yolk, though the structure of the yolk sac is present. The yolk sac is a bilaminar structure with an outer splanchnic mesoderm and inner extraembryonic endoderm. The yolk sac is continuous with the embryonic midgut.

All primordial germ cells, male and female, are derived from yolk sac endoderm. Development is initiated in the early epiblast, where factors such as BMP-4 transform the endodermal cells prior to their migration to the yolk sac. The cells cluster in the caudal surface of the yolk sac mesoderm, close to the primitive hindgut. From that position, they migrate to the wall of the hindgut through the allantoic stalk. Finally, the cells migrate through the dorsal mesentery to reach the genital ridges. It generally takes about three weeks for the cells to make it to the genital ridges, and about 2000 primordial cells ultimately survive the migration. Improper migration is extragonadal regions can result in teratomas. The extent of migration for these primordial germs cells is surpassed only by the migration of neural crest cells.[8]

While not of germ cell lineage, another common condition seen in a laboratory animal, specifically most commonly in the Fischer F344 rat, are endometrial stromal polyps. These occur singularly or as multiples, are usually pale tan or mottled red and tan, and are sessile with a long stalk. They are composed predominantly of loosely organized endometrial stromal cells, vascular supply, and few entrapped glands. The surface epithelium may either be similar to normal endometrial epithelium and low cuboidal or may exhibit squamous metaplasia. When there is an adenomatous component, the terms glandular or adenomatous polyp has been used.[4] These must be distinguished from endometrial stromal sarcoma, which may also be polypoid, but exhibits invasion, poor differentiation of spindle cells, numerous mitotic figures, and high cellular pleomorphism.[3]

Cystic endometrial hyperplasia is one of the most common changes seen in intact female animals, including domestic and laboratory animals, humans, and exotic and wildlife species. It has been reported in nondomestic swine, red wolves, African wild dogs, raccoons, Asian elephants, and wild rabbits. While there is hyperplasia of components of the endometrium, there is prominent hyperplasia of glandular structures, with glands becoming cystic and often detectable macroscopically. A variant also exists where endometrial glands are not cystic and not grossly visible, but there is proliferation of glands (noncystic endometrial hyperplasia).[16] Prolonged and excessive exposure to estrogen is responsible in some species but may also be caused by ingestions of certain plants, or iatrogenic exposure to estrogen containing creams.

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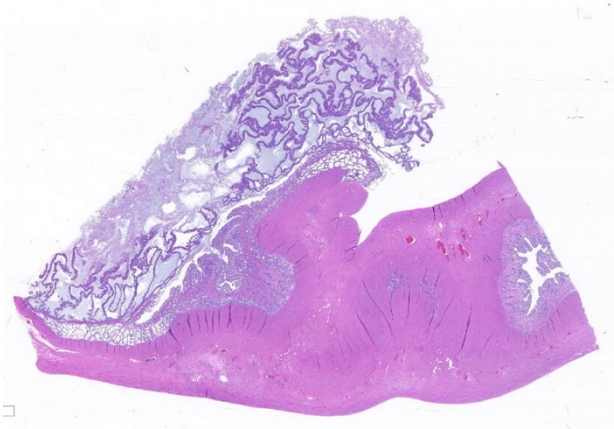
CASE 4: P17-0217 (4100658-00)

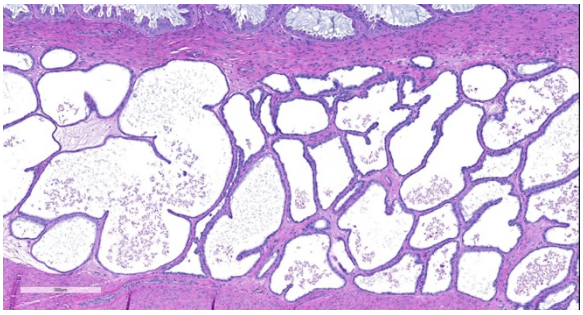
Signalment: 1-year old female spayed Beagle

History: The dog was spayed, and the uterus and ovaries were submitted for histopathology.

Gross Pathology: The uterus contains a 3 cm diameter well circumscribed mass.



Uterus, dog. A sagittal section of uterus is submitted for examination. There is a distinctly layered architecture to the endometrium. (HE, 5X)



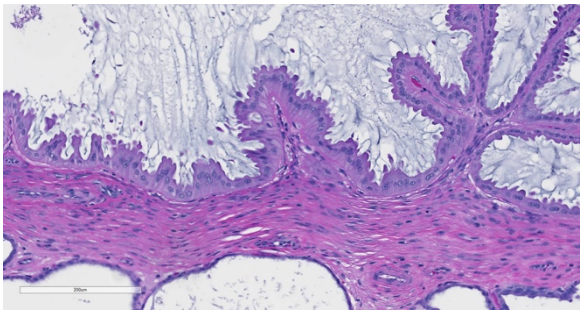
Uterus, dog. A layer of dense connective separates the stratum spongiosum (bottom) from the intermediate zone (top).

Microscopic description: The endometrium has hyperplasia of the surface epithelium forming long fronds extending into the lumen with secondary branching and papillary projections. The epithelium is a single layer of columnar cells with abundant amounts of eosinophilic cytoplasm and basal-oriented nuclei. The lumen is filled with mucus that permeates between the fronds of epithelium. The endometrial glands have hyperplasia and ectasia. Inflammation is lacking.

Contributor's morphologic diagnosis:
Pseudo-placentational endometrial hyperplasia

Contributor's comment: Endometrial hyperplasia in the dog occurs in two forms. The more common form is called cystic endometrial hyperplasia and is a hyperplasia of the endometrial glands. It is frequently associated with pyometra. The other form occurs uncommonly and is called by various names including pseudo-placentational endometrial hyperplasia, deciduoma, and segmental endometrial hyperplasia. Both forms occur under the influence of progesterone from persistent corpora lutea.

The gross and microscopic appearance of the lesion of pseudo-placentational endometrial hyperplasia resembles the normal placental sites in the dog uterus and is the genesis for the naming of the disease.[4,5] The dog with this disease is not pregnant, and the cause of the disease is unknown. The condition has been induced by insertion or injection of a variety of materials within the uterus.



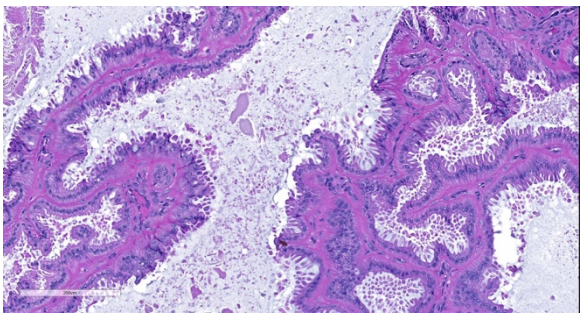
Uterus, dog. The intermediate zone consists of markedly dilated often open-ended glands containing pseudostratified columnar epithelium with apical blebs and glands contain a basophilic mucinous matrix. (HE, 181X)

Contributing Institution:
College of Veterinary Medicine
Virginia Polytechnic Institute and State University
Blacksburg, VA 24061

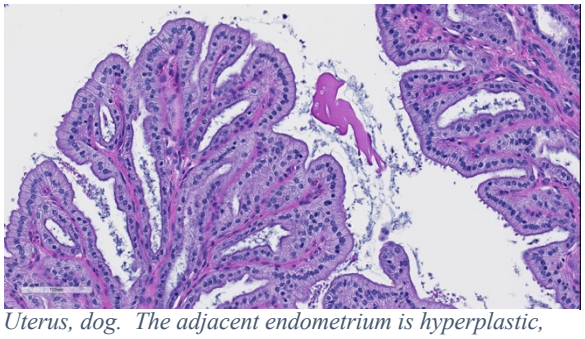
JPC diagnosis:
Uterus: Hyperplasia, endometrial, pseudo-placentational, segmental, Beagle, canine.

JPC comment:
In the bitch, the normal estrous cycle includes differentiation and proliferation of endometrial glands under the influence of estrogen during proestrus, with more extensive proliferation during estrus and metestrus/diestrus under the influence of progesterone. When progesterone levels fall during late diestrus, the endometrium regresses to a quiescent state during anestrus.[3]

There is debate among authors whether cystic endometrial hyperplasia (CEH) and pyometra are linked, it is currently thought that prolonged exposure to high levels of progesterone cause



Uterus, dog. The base layer of the endometrium (stratum spongiosum) is composed of dilated glands lined by cuboidal epithelium. (HE, 81X)



Uterus, dog. The adjacent endometrium is hyperplastic, thrown into papillary projections, and lined by vacuolated (luteinized) columnar epithelium. (HE, 211X)

endometrial gland proliferation which may increase susceptibility of the uterus to infection.[1,3] Three forms of endometrial hyperplasia have been described in the bitch, in addition to CEH-pyometra complex: 1) estrogen-induced CEH, 2) focal endometrial polyps, and 3) endometrial hyperplasia associated with pseudopregnancy (PEH, this case entity).

Histologically, PEH is distinguishable from CEH by only involving a focal or multifocal segment of the uterus, mimicking placentation sites. CEH is typically a generalized diffuse reaction involving the entire endometrium.[5] PEH masses have the three distinct zone of normal maternal placenta, the stratum spongiosum, a zone of dense connective tissue, and the luminal pseudostratified epithelium with multifocal necrotic syncytia formation.[2]

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