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CASE I – 04A486 (AFIP 2987468)

Signalment: 17 day old male Indian Rhesus macaque (*Macaca mulatta*).

History: This animal was born within the Tulane National Primate Research Center colony. Following culture of *Campylobacter coli* from a rectal swab taken at 2 days of age, the infant was transferred to the nursery for care at 3 days of age. Fluid stool, poor appetite and severe dehydration were observed clinically at 17 days of age and the animal died despite receiving subcutaneous and oral fluid supplementation.

Gross Pathology: The body was in good condition with adequate fat stores, but subcutaneous tissues were tacky and the eyes sunken interpreted as dehydration greater than 15% of body weight. The stomach contained approximately 20 ml of curdled ingesta mixed with a clear watery fluid. The small intestine was markedly dilated with clear watery fluid mixed with small amounts of ingesta (Figure 1). The colon was empty and the luminal surfaces were dry. The esophagus contained a small amount of ingesta similar to that found in the stomach. The oral and nasal cavities contained a small amount of curdled ingesta.

Laboratory Results: *Campylobacter coli* was isolated from a rectal swab 1 day prior to transferal to the nursery. *Campylobacter coli* and *Escherichia coli* were isolated from intestine at necropsy. No *Shigella*, *Salmonella*, or *Yersinia* were isolated at necropsy. The *E. coli* culture was submitted to the Gastroenteric Disease Center, Pennsylvania State University, University Park, PA, USA (<http://ecoli.cas.psu.edu>) for typing. The results are presented in the table below.

TABLE

Abbrev	Description	Result
O Type	Somatic antigen	O55
H Type	Flagellar antigen	H7
LT	Heat labile toxin	Negative
STa	Heat stable toxin a	Negative
STb	Heat stable toxin b	Negative
SLT1	Shiga-like toxin I	Negative
SLT2	Shiga-like toxin II	Negative
CNF1	Cytotoxic necrotizing factor 1	Negative
CNF2	Cytotoxic necrotizing factor 2	Negative
EAE	Intimin	Positive
BFP	Bundle forming pili	Negative

Histopathologic Description: Colon and small intestine are submitted. The small intestine has moderate autolytic changes, however lesions are still evident. There is marked villus atrophy and fusion with crypt regeneration and increased numbers of mitotic figures in the crypts (Figure 2). Segments of intestinal epithelium have numerous plump short rods adherent to their apical surfaces (Figure 3). These cells are often attenuated and are sometimes vacuolated. Adherent bacteria are most frequently seen in the ostia to the crypts. The bacteria are Gram negative (Figure 4) and readily demonstrable by Giemsa staining (Figure 5). The colon also has moderate autolytic change, but within the lumen are numerous teardrop-shaped to oval protozoa 5-20 um in length and 3-7 um in width, with a single eccentric nucleus which colonize the surface and superficial glandular lumina (Figure 6). The morphology of the protozoa is consistent with a trichomonad species. There is little to no tissue reaction to the trichomonads.

Contributor's Morphologic Diagnosis: Small intestine: Villus atrophy and fusion with epithelial degeneration and colonization of epithelium by short rods.

Colon: Marked colonization by trichomonads.

Contributor's Comment: The marked villus atrophy and fusion, in the presence of adherent gram negative rod bacteria, is characteristic of attaching and effacing *E. coli* (AEEC) infection. This is consistent with culture results of an *E. coli* strain carrying the intimin (*eae*) gene. Sestak and colleagues found up to 25% of animals at the Tulane National Primate Research Center (TNPRC) were carriers of *E. coli* strains with enteric virulence genes, regardless of the presence or absence of diarrhea (1). However, enteropathogenic *E. coli* (EPEC) infections are considered significant causes of diarrhea and wasting in macaques with AIDS (2). The historically high incidence of previous episodes of diarrhea in the healthy TNPRC cohort (32%) (1) may reflect previous clinical disease associated with EPEC strains which are endemic to the colony.

E. coli causes urinary tract infections, septicemia, and enteric disease (3). Classification of *E. coli* strains is partly dependent upon the somatic (O), flagellar (H), and capsular polysaccharide (K) antigens which are associated with specific clinical syndromes but are generally not virulence factors themselves. For enteric *E. coli* infections, a number of toxins and colonization factors have been identified and are associated with specific clinical syndromes, outlined in the table below. Some of these factors are carried on pathogenicity islands in the bacterial chromosome (e.g. type III secretion systems, *Tir*, *eae*, *espB*), while others are carried on virulence plasmids (e.g. ST, LT, BFP) or have phage-delivered virulence sequences (e.g. Shiga toxin).

Bonnet macaques (*Macaca radiata*) have been shown to be susceptible to experimental inoculation with enterohemorrhagic *E. coli* with an O157:H7 strain, resulting in attaching-effacing lesions with epithelial degeneration and neutrophilic infiltration without fecal blood (5). Examination of fecal samples from healthy and diarrheic new world monkeys (Black tufted ear marmoset, *Callithrix penicillata*; Common marmoset, *C. jacchus*; White-fronted marmoset, *C. geoffroyi*; Saddlebacked tamarin, *Saguinus fuscicollis*; and a Brown howler monkey, *Alouatta fusca*) yielded several *E. coli* strains carrying intimin genes with and without the bundle forming pilus (BFP)(6, 7). Intimin-positive *E. coli* were also isolated from cotton-top tamarins (*Saguinus oedipus*) and associated with active colitis (8). *E. coli* carrying the intimin gene with the BFP are considered "typical" enteropathogenic *E. coli* (EPEC), whereas those without BFP are termed "atypical" EPEC (9). In the case presented here, BFP was not expressed and it would be classified as atypical EPEC.

The serotype of this case (O55:H7) is unlike any EPEC isolates reported from New World monkeys (6, 7), but is instead a strain associated worldwide with infantile diarrhea which is believed to have given rise to the O157:H7 enterohemorrhagic *E. coli* strain (10). We have previously found intimin expression in macaques within

our colony (1), indicating EPEC is endemic. However, we cannot exclude the possibility that, in the setting of the nursery, a nosocomial infection occurred.

The overgrowth of trichomonads in the colon of this monkey was not associated with lesions in the colon. However, trichomonads, tentatively identified as *Tritrichomonas mobilensis*, are opportunistic pathogens and have been reported to invade mucosal sites during AIDS (11).

Abbrev	Name	Virulence factors	Disease association and pathogenesis comments
ETEC	Enterotoxigenic <i>E. coli</i>	Heat-stable toxins (STa, STb) Heat-labile toxins (LT-I, LT-II) colonization fimbriae (K88, K99)	diarrhea in neonates (human, pig, calf, lamb) diarrhea in non-immune adults (traveler's diarrhea)
EHEC	Enterohemorrhagic <i>E. coli</i> (typified by O157:H7) EHEC is a subset of <i>E. coli</i> strains producing Shiga toxins (STEC) which are also termed vero cytotoxins (VTEC)	Shiga(-like) toxins (Stx1 and Stx2) Heat stable toxin (EAST1) in many strains Intimin (eae) chuA (<i>E. coli</i> heme utilization gene) enterohemolysin (pathogenetic effects uncertain)	bloody diarrhea which may be followed by hemolytic uremic syndrome (acute renal failure, thrombocytopenia, microangiopathic hemolytic anemia) Shiga-like toxin effects predominate (hemorrhage and edema of lamina propria), but attaching/effacing lesions occur early
*EAEC or EAggEC	Enteroaggregative <i>E. coli</i>	fimbriae (AAF/I, AAF/II) and a heat stable toxin EAST1 may play a role in pathogenesis	diarrhea in children and healthy adults with excess mucus production
*DAEC	Diffusely adherent <i>E. coli</i>	AIDA-I, an outer membrane protein	watery diarrhea in children 1-5 yrs and possibly nosocomial infections
EPEC	Enteropathogenic <i>E. coli</i>	bundle-forming pilus (BFP) a type III secretion system (sep, esc) and Tir (translocated intimin receptor) Intimin (eae) EPEC-secreted proteins (EspA, EspB, EspD) A heat-stable toxin (EAST1) in some EPEC strains	diarrhea in neonates (humans, rabbits, calves, pigs, dogs) but only very rarely in older hosts attaching and effacing histopathology with cytoskeletal rearrangement increased chloride secretion and enterocyte permeability due to pathologic signal transduction in the enterocyte and malabsorption contribute to diarrhea

*EIEC	Enteroinvasive <i>E. coli</i> Very similar to Shigella spp.	pInV plasmid encoding mxi, spa (type III secretory apparatus) and IpaA, IpaB, IpaC, IpaD invasiveness factors	Initially watery diarrhea which becomes scant and bloody Lesions similar to Shigella, colonic ulceration
NTEC	Necrotoxigenic <i>E. coli</i>	cytotoxic necrotizing factor (CNF1, CNF2)	extraintestinal infections, diarrhea isolated from humans, cattle, pigs, sheep, and dog

*EAEC, DAEC, and EIEC are human pathogens only. EPEC and EHEC together cause attaching/effacing lesions and are also termed attaching and effacing *E. coli* (AEEC)

References: 3, 4

AFIP Diagnoses: 1. Small intestine: Villus atrophy and fusion, diffuse, marked, with enterocyte degeneration and myriad adherent short bacilli, Rhesus Macaque (*Macaca mulatta*), primate.

2. Colon, lumen and glands: Myriad trichomonads.

Conference Comment: The contributor provides a comprehensive review of *E. coli* infection in non-human primates and how different strains of *E. coli* are classified.

A simplified pathogenesis for AEEC begins with the loose attachment to enterocytes by a plasmid-encoded adhesin (EPEC adhesive factor, EAF) which binds via a cell surface glycoprotein receptor, possibly fibronectin. The next step involves the translocation of bacterial proteins into the cytosol of the host cell resulting in cytoskeletal reorganization, effacement of microvilli and formation of a pedestal of cytoplasm that protrudes from the enterocyte and cups the bacteria. Intimate attachment is mediated by a surface-exposed bacterial outer membrane protein called intimin which binds to a receptor called the translocated intimin receptor (Tir) which was originally translocated into the host cell along with other bacterial proteins and has since undergone structural changes. Finally, invasion of host cells is mediated by EAF and the attaching and effacing (*eae*) gene. Invasion is generally more profound with EPEC strains than with EHEC strains (11).

Causes of diarrhea in non-human primates include:

- *E. coli* (ETEC, EPEC, EHEC)
- Shigellosis [*Shigella flexneri* (most common), *S. dysenteriae*, *S. boydii*, and *S. sonnei*]: lesions limited to large intestine; colonic edema, hemorrhage and ulcers +/- pseudomembrane

- Campylobacteriosis (*Campylobacter jejuni* and *C. coli*): similar to shigellosis but also affects small intestine; necrosis, edema, hemorrhage, villus blunting and fusion
- Salmonellosis (*Salmonella enteritidis* and *S. typhimurium*): fibrinonecrotic enteritis, typhlitis and colitis
- *Yersinia enterocolitica*: multifocal intestinal mucosal necrosis, ulcers, hemorrhage, marked neutrophilic inflammation, and large, lobulated bacterial colonies
- *Y. pseudotuberculosis*: acute, severe, fibrinonecrotic enteritis, and mesenteric lymphadenitis with necrosis in the liver, lung, and spleen
- *Proteus vulgaris* and *P. morgani*
- *Cryptosporidium parvum*: protozoa usually line apical border of small intestine enterocytes
- Pinworms: *Oxyuris* sp., *Trypanoxyuris* sp., *Enterobius* sp.
- *Balantidium coli*: ulcerative colitis
- Stress, dietary, inflammatory bowel disease

An excellent review article titled "Attaching-effacing Bacteria in Animals" was recently published in the Journal of Comparative Pathology (11).

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CASE II – 2 (AFIP 2987460)

Signalment: 11-year-old female Franche Montagne Horse (*Equus caballus*).

History: The mare was submitted to the horse clinic, veterinary hospital of the University of Berne, Switzerland with symptoms of therapy resistant fever for 4 weeks, intermittent severe diarrhea and severe ascites. The animal was euthanized.

Gross Pathology: Gross examination revealed approximately 10 liters of clear, yellow fluid in the thoracic cavity and 20 liters of similar fluid in the abdominal cavity (pleural and abdominal effusion). The parietal and the visceral pleura showed areas of numerous, raised, firm reddish nodules (from 0.1 cm up to 1.5 cm diameter). The lung had a thick, fibrotic surface and multifocal to confluent reddish nodules. Inside the abdominal cavity, the ascending colon, the liver, the mesenterium and the diaphragm had thick surfaces and multifocal to confluent reddish nodules.

Laboratory Results: Direct microscopic examination of abdominal fluid revealed mesothelial cells and mitotic figures.

Histopathologic Description: Mesenterium: Expanding the tissue is an unencapsulated, poorly demarcated, infiltratively growing neoplasm composed of

nests and bundles of neoplastic mesothelial cells which occasionally palisade along or form papillary projections on the serosa supported by a large amount of fibrovascular stroma. Neoplastic cells are cuboidal to polygonal to spindle shaped with distinct cell borders. They have a moderate amount of pale eosinophilic fine fibrillar cytoplasm and one round to oval nucleus with finely stippled chromatin and up to two prominent nucleoli. There is moderate to severe anisocytosis and anisokaryosis and occasional multinucleated neoplastic cells. There is less than one mitotic figure per 10 high power fields. Large numbers of lymphatics are dilated and filled by neoplastic emboli. Rarely, on some slides, blood vessels are filled with neoplastic cells, which form papillary projections in the lumen and are admixed with fibrin. Multifocally endothelial cells lining blood vessels are plump (reactive). Multifocally there are foci of necrosis, increased clear space (edema), hyperemia, extravasated erythrocytes (hemorrhage) and multifocal infiltration by few degenerate neutrophils, lymphocytes and plasma cells.

Lung: the serosal surface is severely thickened due to fibrous tissue and infiltration by neoplastic mesothelial cells; multifocally the underlying interstitium is severely thickened by proliferation of fibrous tissue that is infiltrated by the same neoplastic cells. Neoplastic cells also fill alveolar spaces and alveolar capillaries. Multifocally tumor emboli are found also in larger blood vessels, occasionally accompanied by fibrin thrombi. Within alveolar spaces there are scattered foamy macrophages, edema fluid and fibrin.

Contributor's Morphologic Diagnoses: Lung and mesenterium: Malignant mesothelioma, multifocal, Franche Montagne, equine.

Contributor's Comment: Mesotheliomas are primary tumors arising from the mesothelial cells lining the body cavities (thoracic, peritoneal and pericardial cavities) (4). Cases of involvement of the tunica vaginalis of the testis have been reported also. They are rare neoplasms of low grade malignancy with rare metastasis (usually via exfoliated cells). In domestic animals they have greatest frequency in cattle and dogs. They occur most frequently as a congenital neoplasm in fetal or young cattle. Actually, cattle and sheep develop tumors in fetal, newborn and young animals whereas in others species, adult or aged animals are affected. In cattle and goats the location is most commonly peritoneal; in swine mainly pleural. In dogs, the pleura is the main site of development after the pericardium and the peritoneal cavity. Mesothelioma arising from the tunica vaginalis is one of the most common tumors of the male Fischer 344 rat.

Mesotheliomas are rarely seen in horses in which they are reported as mostly malignant, usually pleural and pericardial but also abdominal (1, 2, 3, 5).

Mesotheliomas are also reported in buffalo, mice, fowl, hamsters, and cats.

In humans, an association between malignant mesothelioma and asbestos exposure has been well established. In domestic animals, they generally occurs

spontaneously. Ferruginous bodies (fine fibers coated with amorphous protein and ferritin) which are suggestive of asbestos exposure, have been identified in the lungs of urban dogs with mesothelioma (this association is confirmed in humans). However there is little evidence for a relationship between asbestos inhalation and mesothelioma. Concerning newborn cattle, a congenital origin has been found for mesotheliomas.

The clinical signs associated with mesothelioma are typically anorexia and depression, dyspnea, respiratory distress, cardiac insufficiency, cardiac tamponade, decreased lung sounds, and/or abdominal distension (accumulation of large amounts of fluid, resulting in ascites) depending upon the location of the tumor. On gross examination, tumors are formed either by small nodules, sessile or pedunculated, from a few millimeters to 10 centimeters in diameter or by villous projections arising from the serosal surface. Depending on the amount of hemorrhage, the color varies from gray-white to red. Some fibrous or sclerosing forms have been reported as well as milky or blood-tinged effusion. Ascites if the peritoneum is involved and adhesions may also be present.

Mesothelioma can be papillary epithelioid or sarcomatoid or, most commonly, a combination of these two patterns (biphasic). Generally cells are pleomorphic (polygonal to spindle-shaped), occasionally multinucleated and display anisokaryosis with a prominent nucleolus. Neoplastic cells can line cystic spaces and tubular structures which may contain a mucinous matrix. Cells line papillary structures in several layers. Mitotic figures are rare. In the epithelioid form, cuboidal epithelioid cells cover papillary projections made of spindle-shaped cells and conjunctivo-vascular stroma. Sometimes cells can form tubules or rosettes and mitoses are usually not numerous. Sclerosing mesotheliomas are composed of large anaplastic cells with sometimes multinucleated giant cells. (6)

Mesotheliomas are of low grade malignancy so there is limited invasive growth. Metastases to drainage lymph nodes are rare and distant metastases very rare. It is not always possible to determine whether widespread involvement of the peritoneal cavity and the tunica vaginalis is the result of secondary spread or of multicentric origin. (6)

Concerning the epithelioid pattern, the differential diagnosis must include metastasis from an adenocarcinoma located in another site, mammary gland or ovary for examples. Lipomas, liposarcomas and more rare tumors such as ganglioneuromas can also develop from the serous membranes.

Ultrastructurally, neoplastic mesothelial cells have a prominent basal lamina, many long slender, well-developed microvilli present on all surfaces, prominent desmosomes, abundant rough endoplasmic reticulum, and mitochondria.

Immunohistochemical stains may be helpful in differentiating mesothelioma from other neoplasms. Mesothelioma are positive for vimentin, cytokeratin 5, LP 34; they are negative for CEA (carcinoembryonic antigen), CD 15 (LEU M1), BER EP4 (tumor glycoprotein) (markers for neoplasm of epithelial origin), S100, HMB 45 (markers for neoplasm of melanocytic origin).

The mucicarmine stain with and without hyaluronidase can help distinguish mesothelioma from adenocarcinoma. The presence of mucicarmophilic, hyaluronidase resistant material within cytoplasmic vacuoles supports adenocarcinoma. By immunohistochemistry, neoplastic cells of mesothelioma are typically positive for both keratin and vimentin. Carcinomas are usually keratin positive and vimentin negative.

Calretinin was also used as an immunohistochemical marker in the diagnosis of mesothelioma in an equine species (7: Stoica G. et al. 2004).

Immunohistochemically, in the present case, the neoplastic cells are multifocally positive for keratin and occasionally positive for vimentin. Thus, histomorphology and immunohistochemistry support mesothelioma. The calretinin immunohistochemical stain is running. The widespread involvement of the peritoneal cavity and the involvement of the lung parenchyma render this case particularly rare and interesting.

AFIP Diagnoses: 1. Mesentery (per contributor): Malignant neoplasm, with vascular invasion, and marked sclerosis, Franche Montagne horse, equine.

2. Lung, alveolar capillary and arterial lumina: Malignant neoplasm, with thrombi, infarcts, edema, and marked interlobular and pleural fibroplasia.

Conference Comment: This neoplasm has proven to be a challenge to both describe and diagnose. Conference attendees debated everything from tissue identification and cell shape to immunohistochemical interpretations. The section of mesentery was simply identified as fibroadipose tissue and the arrangement of neoplastic cells did not easily fit into any of the three recognized patterns for mesotheliomas (i.e. papillary epithelioid, sarcomatoid or biphasic). Several attendees recognized the numerous pulmonary vessels filled with tumor cells and considered a round cell neoplasm such as lymphoma. Others favored widespread carcinomatosis as a differential. All agreed neoplastic cells express strong cytoplasmic immunopositivity for pancytokeratin; however, both the AFIP's vimentin and calretinin results are equivocal. All agreed that electron microscopy to identify desmosomes and microvilli on the cells would be very helpful in differentiating carcinoma from mesothelioma. Regardless, the contributor provides an excellent, detailed review of mesotheliomas and a challenging neoplasm to spark much debate.

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CASE III – 05040376-2,3 (AFIP 2985397)

Signalment: Yearling mixed breed bovine

History: During late winter/early spring of 2005, increased morbidity and mortality was reported in range cattle on the Navajo Reservation in northeast Arizona. Clinical signs included: staggering, stupor, blindness and tongue paralysis. Yearlings and two year olds were principally affected. Tansy mustard (*Descurainia pinnata*) was in exuberant bloom on the range.

Gross Pathology: Field necropsies of affected animals revealed no significant gross lesions.

Laboratory Results: Toxicological analysis:

Sample:	Tansy mustard, leaves and stems
Selenium	0.48 ppm
Sulfur	9410 ppm
Nitrate	2392 ppm

Histopathologic Description: Multiple sections of cerebrum, cerebellum and brainstem are examined and significant lesions are restricted to the cerebral cortex. There are disseminated foci of laminar cortical necrosis of neurons characterized by shrunken, hypereosinophilic neurons that exhibit either karyorrhexis, karyolysis or complete loss of nuclear detail. Regionally, the foci of necrosis are accompanied by mild to marked populations of astrocytes (astrogliosis) and rarefaction of the neuropil. Both sulci and gyri are affected.

Contributor's Morphologic Diagnosis: Brain, cerebral gray matter: Severe laminar cortical necrosis with prominent astrogliosis

Contributor's Comment: *Descurainia* spp. plants are native to most regions of North America and are present in open prairie, fields and along roadways. Several species of plants in this family are potentially toxic to animals and *Descurainia pinnata* (tansy mustard) associated with "tongue paralysis of cattle" is the most well known, see review by Burrows (1).

Cattle are the principle species affected in reported outbreak situations; however, sheep, horses and goats may also be affected. Toxicities under range conditions follow winters of over-abundant moisture. In this case, abundant plant growth was reported because of above normal autumn and winter rainfall.

Historically, intoxicated cattle present initially with varying degrees of blindness, walking in circles or become difficult to drive. Later tongue paralysis develops, and the early reports of outbreaks utilized this as the syndrome name. Following tongue paralysis, the animals have difficulty obtaining feed or water and become weak and depressed. Spasms or tremors of muscles of the head, ear or neck (including head bobbing) have also been described in cattle, but are more prominent signs in horses and goats. Animals die from weakness and inanition; however, many recover with removal from contaminated pastures and with medical treatment. In the currently reported outbreak, many animals that were removed from the pastures and/or treated with thiamine clinically improved.

Early suspicions for the toxic principle for *Descurainia pinnata* were centered around selenium accumulation; however, selenium levels have not been found to be elevated in this plant. Elevated sulfur levels have been previously described in tansy mustard (2) and confirmed by analysis of leaves and stems from the pasture plants implicated in this case. Sulfur is known to cleave and render thiamine inactive and the positive response to thiamine treatment suggests that sulfur-induced thiamine inactivation may play at least some role in the pathogenesis of the disease. The central nervous system clinical signs associated with this plant have not yet been experimentally produced, so the exact mechanism remains a mystery.

AFIP Diagnosis: Brain, cortical grey matter: Necrosis, laminar, multifocal, with gliosis, mixed breed, bovine.

Conference Comment: Several saline tolerant plant species are known to accumulate sulfur. A short list includes: *Kochia scoparia* (burning bush, fireweed), *Descurainia pinnata* (tansy mustard), *Melilotus officinalis* (yellow sweet clover), *Elymus trachycaulus* (slender wheatgrass) and *Helianthus annuus* (sunflower)(2). Inorganic sulfur compounds are capable of cleaving thiamine and rendering it inactive and high levels of sulfates in the diet or drinking water have been shown to increase the incidence of polioencephalomalacia in cattle and sheep(4).

Thiamine (Vitamin B1) deficiency is a common cause of progressive encephalopathy that affects young ruminants and carnivores at any age. Thiamine deficiency is also known as polioencephalomalacia (PEM), cerebrocortical necrosis, Chastek paralysis (in carnivores), forage poisoning and blind staggers.

Ruminants depend on ruminal microbial synthesis of thiamine. Deficiency may be caused by grain overload with the subsequent overgrowth of thiaminase producing bacteria such as *Bacillus thiaminolyticus*, *Clostridium sporogenes* and *Bacillus aneurinolyticus*. During absorption, thiamine is phosphorylated to produce thiamine pyrophosphate which has three main functions: 1) it regulates the synthesis of ATP; 2) acts as a cofactor for transketolase, an important enzyme in the Pentose Phosphate Pathway (PPP), which is the primary metabolic pathway for central nervous system glucose metabolism; and 3) it maintains neural membranes and normal nerve conduction (3). Additionally, thiamine pyrophosphate is a cofactor for alpha-ketoglutarate dehydrogenase, pyruvate dehydrogenase, branched-chain alpha-keto acid dehydrogenase, and probably contributes to most oxidative decarboxylations and metabolic transformations. Transketolase is also apparently important in the metabolism of oligodendrocytes (4).

Other causes of polioencephalomalacia related to thiamine deficiency in ruminants include the ingestion of thiaminase-containing plants such as bracken fern (*Pteridium aquilinum*), horsetails (*Equisetum arvense*), and nardoo (*Marsilea drummondii*), also molasses in feedlot cattle, and the administration of thiamine antagonists such as amprolium.

Carnivores, unlike ruminants, have a dietary requirement for thiamine and deficiency can be caused by reduced intake. Additionally, foods high in thiaminase, such as tuna, salmon, and meats that have been exposed to excessive heating and preservation by sulfur dioxide can all result in thiamine deficiency.

In ruminants, the characteristic microscopic lesion of polioencephalomalacia is laminar cortical necrosis. Once laminar cortical necrosis is recognized, it should bring to mind a differential diagnosis which includes:

1. Polioencephalomalacia (thiamine deficiency)
2. Sulfur toxicosis (accumulation in plants or high levels in water, resulting in thiamine deficiency)
3. Lead poisoning (also see basophilic stippling of erythrocytes and increased nucleated red blood cells, > 5/100 WBC)
4. Salt poisoning/Water intoxication (following water deprivation)
5. Hypoxia (due to peri-mortem agonal breathing)

Interestingly, approximately one-fourth of chronic alcoholics admitted to general hospitals suffer from thiamine deficiency. In humans, thiamine deficiency predominantly affects the peripheral nerves, heart and brain. Polyneuropathy associated with thiamine deficiency is known as dry beriberi and results in myelin degeneration; whereas, the condition affecting the cardiovascular syndrome is called wet beriberi and results in a flabby, dilated heart. The most advanced form is known as Wernicke-Korsakoff syndrome and is characterized by ataxia, confusion, apathy, listlessness, disorientation, nystagmus, ophthalmoplegia, retrograde amnesia, the inability to acquire new information, and confabulation (3). Some would argue that these are also the classic signs of an overwhelmed pathology resident and that perhaps they should all start thiamine supplementation.

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CASE IV – 05-13561B (AFIP 2984526)

Signalment: 57 day fetus, intact male, Shih Tzu, *Canis familiaris*, canine

History: The breeding kennel had three late term abortions in the last month. The submitted fetus had been aborted approximately seven days before the expected whelping date. The kennel had a history of *Brucella canis* and has had an extensive serologic testing and culling program in progress for the last 4 years.

Gross Pathology: There were no gross abnormalities.

Laboratory Results: Microbiology: Aerobic cultures of the kidney and liver were negative. There was a moderate, pure growth of *Brucella canis* from the lung on aerobic culture. There was a heavy growth of *Brucella canis* from placenta on aerobic culture. The bacterial species was confirmed by the Minnesota Department of Health by biochemical assays.

Serology: Tube agglutination for *Brucella canis* on serum from the dam was considered suspect at 1:200.

Virus isolation: Virus isolation for canine herpesvirus was negative after two passes on MDCK.

Gram stain: The bacteria were Gram negative.

Histopathologic Description:

Contributor's Morphologic Diagnosis: Necrotizing placentitis, moderate, acute, diffuse with myriad intracellular bacteria Shih Tzu, *Canis familiaris*

Contributor's Comment: *Brucella canis* was discovered as a canine pathogen in 1966 by L. Carmichael in Beagles.^{3,4} Since then it has been documented around the world. In the United States the estimated prevalence is 0.5-5%.³ *Brucella* species are Gram negative, aerobic, slow growing coccobacilli.^{2,4} *B. canis* is a mucoid strain, similar to *B. ovis*, which does not express the O antigens that the smooth strains, such as *B. abortus* and *B. melitensis*, do.^{3,4} Because of this, the antigens in commercial test kits for *Brucella* will not cross-react with *B. canis*.³ Dogs can be infected with *B. abortus*, *B. melitensis*, and *B. suis* on an individual animal basis but *B. canis* is an epidemiologically important pathogen in kennels.⁴

Clinical signs in dogs range from asymptomatic to reproductive failure. In bitches, the most common clinical sign is late term abortion, with the majority after 50 days.^{3,4} Other signs include weak born pups that die a few days to one month after birth and rarely pups that live with a persistent infection.^{3,4} After abortion, the bitch will normally have a brown to green vaginal discharge for 1-6 weeks.^{1,4} In males, the most common clinical signs are epididymitis and testicular atrophy with secondary, lick-induced, scrotal dermatitis.^{2,3} Some dogs may develop prostatitis.^{2,3} Prepubertal dogs that are infected may have generalized lymphadenopathy.⁴ Other diseases caused by *B. canis* include diskospondylitis, osteomyelitis, meningoencephalitis, granulomatous dermatitis, and recurrent anterior uveitis.²

The aborted feti are usually partially autolyzed and show changes consistent with a bacterial infection. Combinations of the following are seen in aborted feti: subcutaneous edema, subcutaneous hemorrhage of the abdominal wall, pneumonia, endocarditis, hepatitis, and ascites. Our case was highly unusual in that there were no gross or microscopic abnormalities in the feti, only in the placenta. The placenta had a majority of the trophoblasts filled with abundant, basophilic, small (approximately 1 micron in diameter), coccobacilli with occasional bacterial colonies extending into the mesenchyme. There was also multifocal villus necrosis and infrequent lymphocytic vasculitis. These findings are consistent with published reports.³ Other infectious agents that can cause abortion in the bitch include canine herpesvirus, *Toxoplasma gondii*, and *Neospora caninum*.¹ Canine herpesvirus mainly manifests as pups born live which become ill 1-2 weeks after birth and die.¹ Classical gross lesions include subserosal petechia and ecchymoses and microscopically there should be rare, basophilic, intranuclear inclusion bodies.³ *Toxoplasma gondii* and *Neospora caninum* are protozoal pathogens that rarely cause abortion in dogs, more commonly in sheep and cattle respectively. On microscopic exam, cysts should be seen within the brain.³

After an animal becomes infected with *Brucella canis* the bacteria invade macrophages and spread to lymphoid tissues (lymph nodes and spleen) where they replicate.² Bacteremia occurs 1-4 weeks post infection and persists for a minimum of six months then intermittently up to 5 years.² The bacteria subsequently spread to the target organs (genital tracts) and can cause clinical signs.

Transmission of *Brucella canis* is through mucous membranes, most commonly oral, conjunctival, and vaginal. The bacteria are shed in the aborted fetus, placenta, vaginal discharge, and semen in the highest concentrations but are also secreted in the urine of male dogs and milk.² Bacterial transfer can also occur through fomites such as vaginoscopes, artificial insemination equipment, and syringes, but this mode is less likely since the bacteria do not live long outside the

host.² The bacteria can also be transmitted in utero but most of the pups die and do not become persistently infected.²

The diagnosis of *Brucella canis* is not simple. The gold standard is culture, either of blood from live animals or tissues from necropsy specimens. Other antemortem diagnostics include multiple forms of serology: tube agglutination with or without 2-mercaptoethanol, agar gel immunodiffusion, enzyme linked immunosorbent assays, and immunofluorescence. Serology is not always sensitive or specific and definitive diagnosis is through blood culture.⁴

AFIP Diagnosis: Chorioallantois: Placentitis, necrotizing, acute, multifocal, moderate, with myriad intratrophoblastic coccobacilli, Shih Tzu, canine.

Conference Comment: The contributor provides an excellent review of *Brucella canis* infection as well as other causes of abortion and stillbirth in dogs.

All *Brucella* species are closely related, pathogenic, gram-negative, intracellular rods with a predilection for the reproductive tract and all cause similar diseases characterized by:

- Reproductive failure in young females with persistent lifelong infection and shedding from reproductive tissues and mammary glands
- Epididymitis and orchitis in males
- Bronchopneumonia in aborted fetuses

The ungulate placenta, fetal fluids and testes contain a polyhydric alcohol known as erythritol which has been shown to stimulate growth of *Brucella*. Erythritol is not present in the human placenta. Virulent strains of *B. abortus* release 5' guanosine monophosphate and adenine which inhibit PMN degranulation contributing to intracellular survival. *B. abortus*, *B. melitensis* and *B. suis* have surface antigens A and M present on the LPS protein complex. *B. canis* and *B. ovis* possess an R surface antigen. Since all *Brucella* sp. are intracellular bacteria, acquired immunity is mainly cell mediated.(5)

Below is a simple list to help remember the different species of *Brucella* and what species they most commonly affect:

- **Cattle:** *B. abortus*. Placentitis, abortion, mastitis, epididymitis, orchitis
- **Swine:** *B. suis* (also *B. abortus* and *B. melitensis*). Sterility, abortion, arthritis


- **Sheep & goats:** *B. melitensis*. In goats similar to *B. abortus* in cattle with occasional mortality; less severe in sheep
- **Sheep:** *B. ovis*. (also *B. abortus*) Epididymitis, rarely placentitis and abortion
- **Dogs:** *B. canis*. Prostatitis, epididymitis, testicular atrophy, abortion, osteomyelitis, diskospondylitis
- **Horse:** *B. abortus*, *B. suis*. Suppurative bursitis (poll evil and fistulous withers)
- **Wildlife:** Described in a wide variety of mammals, including ungulates and marine mammals; especially bison, elk, reindeer and other ungulates (*B. abortus*)

Humans are susceptible to all species (most commonly *B. abortus*, *B. melitensis* and *B. suis*), as well as RB51 and strain 19 vaccines. Transmission is by infected milk products or direct contact with infected tissues. Symptoms include prolonged or recurrent fever, weakness and impotence. Brucellosis in humans is known as undulant fever, gastric fever, Malta fever, Gibraltar fever, and Mediterranean fever. *Brucella abortus* is also commonly associated with olecranon bursitis in people.

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