Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2012-2013

Conference 9

28 November 2012

CASE I: 09-1-481 (JPC 3167630).

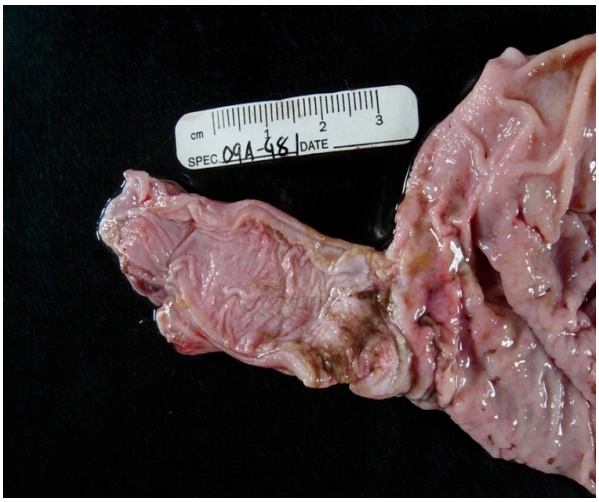
Signalment: 4-year-old female rhesus macaque, *Macaca mulatta*, nonhuman primate.

History: A week before necropsy, the animal received intravenous antibiotic therapy (Cefazolin) following surgical placement of hormonal implants as part of a research protocol. A day before necropsy, the animal was lethargic and was reported to be sitting with head tucked under hind limbs and with loss of appetite. On the day of necropsy, abdominal bloating was noted.

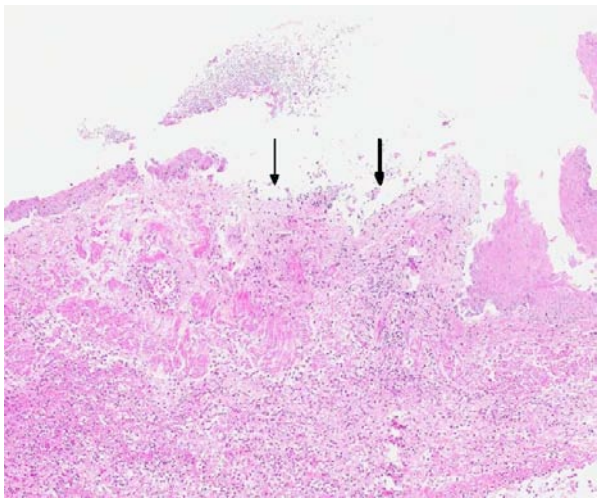
The animal was azotemic and hypoglycemic with marked leukopenia and metabolic alkalosis. The animal had not responded to fluid therapy and was euthanized. *Candida albicans* was isolated from the stomach contents at necropsy.

Laboratory Results:

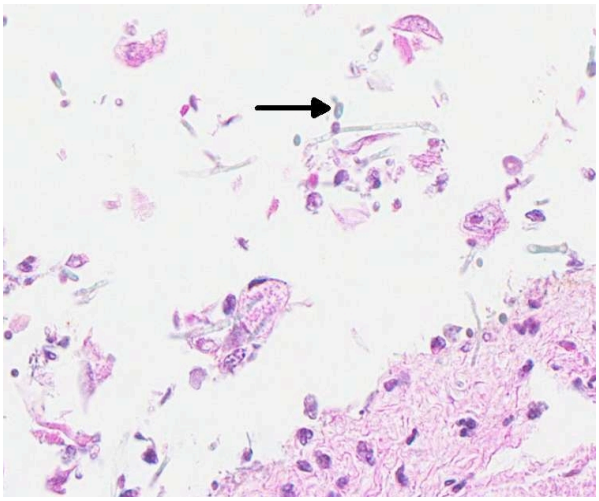
		Reference Range
RBC	6.37 X10^6/mm^3	5.0 - 6.5 X 10^6/mm^3
WBC	2.8 X10^3/mm^3	6.0 - 15.0 X10^3/mm^3
Lymphocytes	19%	25.0 - 60 %
Monocytes	1%	0 – 8 %
Eosinophils	0%	0.0 - 5.0 %
pH	7.304	>7.40
Sodium	150 mmol/L	141-153 mmol/L
Potassium	3.0mmol/L	2.9-4.1 mmol/L
Blood Urea Nitrogen	82mg/dl	16-27 mg/dl
Glucose	34 mg/dl	39-82 mg/dl



1-1. Esophagus, rhesus macaque: The mucosa of the esophagus and gastric cardia are multifocally ulcerated, necrotic, hemorrhagic, and edematous. (Photo courtesy of Oregon National Primate Research Center. http://onprc.ohsu.edu)



1-2. Esophagus, rhesus macaque: The mucosal epithelium is multifocally lost (arrows) with infiltration and expansion of the subepithelial connective tissue and submucosa by a large numbers of neutrophils and lesser numbers of macrophages. (HE 60X)



1-3. Esophagus, rhesus macaque: Within the lumen, mucosa, and rarely the submucosa there are numerous oblong yeasts (arrow) and hyphae, characteristic of Candida species. (HE 400X)

Gross Pathologic Findings: The abdomen was distended and doughy. On opening the abdominal cavity, severe bloating and distension of stomach was noted. The stomach contained about 200 g of partially digested feed material admixed with blood. The mucosa of the esophagus was multifocally ulcerated, thickened with multifocal areas of hemorrhage. The mucosa of the stomach was multifocally ulcerated and hemorrhagic.

Histopathologic Description: The stratified squamous epithelium of the esophagus is multifocally necrotic and ulcerated. There is a focally extensive suppurative focus characterized by the presence of large numbers of viable and degenerate neutrophils disrupting the mucosa, submucosa and extending into muscular tunics and serosal layer. Overlaying and infiltrating the necrotic mucosa are aggregates of numerous oval to round, 3-6 µm diameter, pale staining, thin-walled yeast; blastoconidia arranged in short chains (pseudohyphae); and slender, 3-4 µm wide, septate, parallel-walled, hyphae that often show acute angle branching. Multifocally, the collagen fibers in the submucosa are disrupted by edema and the walls of medium and small sized blood vessels are necrotic and infiltrated by neutrophils.

Contributor's Morphologic Diagnosis: Esophagus: Esophagitis, necrosuppurative, ulcerative, transmural with vasculitis, intralesional mycelia and yeasts consistent with *Candida albicans.*

Contributor's Comment: *Candida albicans* is a normal inhabitant of the nasopharynx, GI tract, and reproductive tract of many species of animals and is opportunistic in causing disease.[2] Predisposing factors include disruption of mucosal integrity, indwelling intravenous or urinary catheters, administration of antibiotics or immunosuppressive drugs and diseases. Activation of virulence factors play a major role in dissemination and colonization in systemic *Candida* infections.[1]

This animal received multiple intravenous antibiotics following surgical implantation of hormonal depots. The gastritis lesions were chronic and the esophageal lesions are attributed to acid reflux and subsequent colonization by *Candida albicans*. In rhesus monkeys infected with simian immunodeficiency virus (SIV), candidiasis is a common opportunistic infection; however, this animal has not been infected with SIV. Also, nonhuman primates are an excellent animal model for studying oral candidiasis.[8,9]

Systemic and cutaneous candidiasis has also been described in cattle, calves, sheep, and foals secondary to prolonged antibiotic or corticosteroid therapy.[4] In cats, candidiasis is rare but has been associated with oral and upper respiratory disease, pyothorax, ocular lesions, intestinal disease, and urocystitis. In dogs, *C. albicans* is reported to cause stomatitis, spondylitis, endophthalmitis and purulent pericarditis.[3,5,6,7] *Candida* spp. has been considered a cause of arthritis in horses and mastitis and abortion in cattle. In birds, the infection causes stomatitis, esophagitis and ingluvitis. In piglets, the infection causes stomatitis, esophagitis and gastritis.[1] In horses, *C. albicans* causes ulcerative gastritis adjacent to margo plicatus.[1]

Candida spp. are pleomorphic, with both yeast and mycelia phases present in tissue. The differential diagnoses include: *Aspergillus* species., which form septate hyphae with dichotomous branching and bears conidiospores; *Zycomycetes* species, which form nonseptate, branching hyphae with bulbous enlargement; *Histoplasma capsulatum*, which are intrahistiocytic yeast; and *Blastomyces dermatitidis,* which are 7-17 µm large yeast with broad- based budding.

JPC Diagnosis: Esophagus: Esophagitis, ulcerative and neutrophilic, with moderate numbers of extracellular yeast and pseudohyphae.

Conference Comment: Conference participants discussed the distinguishing morphologic features of *Candida albicans*, a trimorphic fungus that is one of only three species of *Candida,* along with *C. tropicalis* and *C. dubliniensis,* that occur in three vegetative morphologies: yeast, pseudohyphae, and hyphae. The yeast form, also called blastoconidia or blastospores, are oval, single-celled structures; hyphae and pseudohyphae are filamentous multicellular structures in which elongated cells are attached end-to-end. Psuedohyphae can be differentiated from true hyphae by the following characteristics: Pseudohyphal cell walls are not parallel; rather, they are wider at their center and narrower at their ends, with constrictions at cell junctions. Hyphal cells, on the other hand, have parallel walls, and are more uniform in width, with true septa (internal cross walls that divide the cells). Additionally, true hyphal cells have pores in their septa, allowing for cell-to-cell communication. Although pseudohyphae appear more similar to hyphae microscopically, they are actually more closely related to the yeast form, and thus can be thought of as an intermediate between yeast and true hyphae composed of strings of attached, elongated yeast cells.[10]

Pathogenicity of fungal organisms is related to their morphology. In *Candida albicans,* the single-cell yeast form is thought to be evolutionarily adapted for colonization of mucosal cell surfaces and allows for rapid dissemination via the bloodstream in systemic infections; pseudohyphae are associated with increased virulence properties and enhanced nutrient scavenging; and the formation of hyphae is an important virulence factor which allows the fungus to invade epithelial and endothelial cells and lyse macrophages and neutrophils. The necessity of hyphal formation for pathogenicity is demonstrated by the significant attenuation of virulence in *C. albicans* cells lacking the filament-induced gene HGC1, which drives hyphal development. In addition, several other hyphal-specific genes are also important for pathogenicity. ALS3 and HWP1 encode adhesins, which allow *C. albicans* to leave the circulation, colonize tissue, and form a biofilm. Degredative enzymes such as aspartyl proteinase (SAP) contribute to tissue invasion. SOD5, which encodes a superoxide dismutase that protects against oxidative stress, is also induced during hyphal growth. HYR, another hypha-specific gene, plays a role in neutrophil killing. Thus, the ability of *C. albicans* to form hyphae contributes to their increased virulence compared to other *Candida* species that only form yeast and pseudohyphae.[10]

Contributing Institution: Pathology Services Unit
Department of Animal Resources
Oregon National Primate Research Center
505 NW 185th Avenue
Beaverton, OR 97006

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CASE II: Yn12-31 (JPC 4019363).

Signalment: 4-year-old male rhesus macaque (*Macaca mulatta*).

History: An adult male rhesus macaque was transferred from CRO to YNPRC and assigned to a renal transplantation study protocol. Post-transplant day 38, the monkey showed signs of failure to thrive after immunosuppression was induced by T-cell depletion and steroids, and maintained on anti-rejection drugs. Animal continued to lose weight despite treatment with low dose steroids and antibiotics. Anemia was diagnosed and treatment with iron and B12, and a transfusion of irradiated whole blood (100mL) were given. Anorexia and weight loss continued. Due to poor prognosis the monkey was euthanized.

Gross Pathology: This adult male rhesus macaque weighed 5.20 kilograms. The animal was in a very thin body condition with prominent bony structures. Omental adhesions to the transplanted kidney were present. There were no other gross findings.

Laboratory Results:

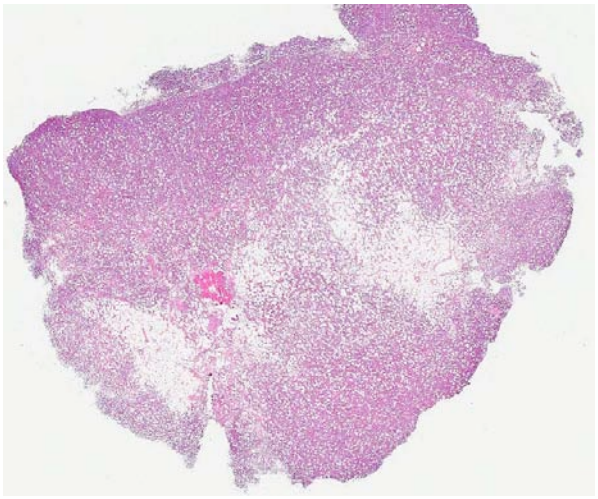
Hematology Parameters	Pre-study	Post renal transplant Day 28	Post whole blood transfusion and Prior to necropsy
RBC - µl	5.31	2.31	2.93
Hemoglobin - gm%	12.8	5.1	6.9
Hematocrit (HCT) - %	37.6	14.9	19.0
Reticulocyte count (RETIC) - % RBC	0.0	0.3	2.7
Mean corpuscular volume (MCV) - fl	70.8	64.5	64.8
Mean corpuscular hemoglobin (MCH) - pg	24.1	22.1	23.5
Mean corpuscular hemoglobin concentration (MCHC) - g/dL	34.0	34.2	36.3
Platelets	377000	373000	619000
WBC	8700	2860	1420
Neutrophils	2436 (28%)	1801 (63%)	908 (64%)
Lymphocytes	6003 (69%)	858 (30%)	454 (32%)
Monocytes	87 (1%)	143 (5%)	56 (4%)
Eosinophils	174 (2%)	57 (2%)	0 (0%)

Histopathologic Description: Microscopic examination of femoral bone marrow revealed hypercellular marrow with abnormal erythroid cells with bizarre nuclear forms and intranuclear inclusions. These cells have a glassy intranuclear eosinophilic inclusion body, stained pink or lilac, that displaces the chromatin to the periphery. Ultrastructural examination revealed intranuclear viral particles and an occasional viral array characteristic of parvoviruses.

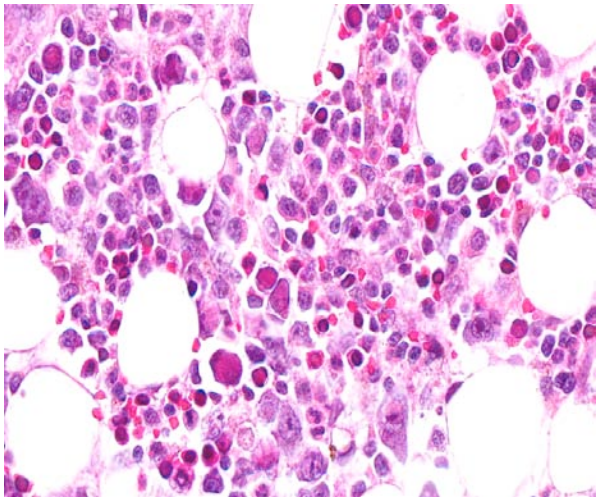
Contributor's Morphologic Diagnosis: Hypercellularity of femoral bone marrow with numerous intra-nuclear viral inclusion of Simian Parvovirus.

Contributor's Comment: *Simian parvovirus* (SPV) is a erythrovirus within the *Parvoviridae* family, related antigenically to human B19 virus.[1,2,4] All of the primate erythroviruses have a predilection for erythroid precursors. The epizoology of SPV is poorly understood, but infection has been recognized in cynomolgus and rhesus macaques.

In humans, B19 can persist at low levels in the bone marrow of infected humans for extended periods, establishing latency, and a similar situation can be anticipated to occur in macaques with the related SPV. Both viruses target rapidly dividing cells and demonstrate a tropism for cells within the erythrocytic lineage. In immunologically normal animals, infection has not been associated with clinical disease, but with immunosuppression or immunodeficiency; infection may cause anemia and widespread infection of erythroid cells. In bone marrow, poorly-defined eosinophilic intranuclear inclusions may be observed, in association with dyserythropoiesis.[4] Ultrastructural examination and *in situ* hybridization can be used to confirm the diagnosis.[4] Such infections and pathology have been observed in both SIV- and SRV-infected rhesus macaques, as well as in immunosuppressed



2-1. Bone marrow, rhesus macaque: The bone marrow is diffusely hypercellular, as evidenced by a subjective decrease in adipose tissue. (HE 20X)



2-2. Bone marrow, rhesus macaque: Erythroid cells outnumber myeloid cells by a 3:1 margin and often contain large intranuclear viral inclusions. (HE 400X)

cynomolgus macaques, in which severe clinical anemia has been diagnosed.[1,4]

A SPV infection can be particularly problematic in transplantation studies in which NHPs received some form of immunosuppressive therapy to prevent transplant rejection. Using a combination of molecular detection techniques such as PCR and serologic testing, monkeys can be screened prior to initiation of studies and the selection of viral-negative animals will help prevent transmission from donor to recipient. However, immunosuppression allows latent infection to manifest itself, as in this case.

JPC Diagnosis: 1. Bone marrow: Erythrocytic hyperplasia, mild to moderate.
2. Bone marrow, erythrocytic precursors: Intranuclear viral inclusions, numerous.

Conference Comment: In addition to discussing simian parvovirus, for which the contributor provided a good review, conference participants also discussed other parvoviruses of importance in veterinary medicine. Viruses in the family *Parvoviridae* are non-enveloped, single-stranded DNA viruses that lack enzymes for DNA replication and thus require host cell DNA polymerase;[3] hence, they replicate in host cell nuclei during the S phase of the cell division cycle. Their need to replicate in cells undergoing cell division determines the pathogenicity of the virus. Infections in fetuses or newborns during organogenesis can lead to serious defects, as the virus destroys developing tissues such as the cerebellum (such as in feline panleukopenia) and the myocardium (such as in canine parvovirus). In older animals, only rapidly dividing cells (hematopoietic precursors, lymphocytes and mucosal cells lining the gut) are affected.[3]

The family *Parvoviridae* is divided into two subfamilies, *Parvovirinae* and *Densovirinae*. *Parvovirinae* contains viruses of vertebrates, and *Densovirinae* viruses affect insects. The *Parvovirinae* subfamily is further divided into five genera: *Parvovirus, Erythrovirus, Dependovirus, Amdovirus, and Bocavirus.*[3] The following tables list parvoviruses of veterinary importance and the diseases associated with them:[3]

Genus *Parvovirus*

Virus	**Disease**
Feline Panleukopenia Virus	Generalized disease in kittens; panleukopenia; enteritis; in-utero or neonatal infection can cause cerebellar hypoplasia; raccoon, mink and coatimundi are also susceptible
Mink Enteritis Virus	Leukopenia; enteritis
Canine Parvovirus 2 (3 major variants: subtypes 2a, 2b, 2c)	Generalized disease in puppies; enteritis; myocarditis; lymphopenia
Porcine Parvovirus	Stillbirth, mummification, embryonic death, infertility (SMEDI); rare respiratory disease, vesicular disease and systemic disease of neonates
Rodent Parvoviruses (Mouse parvoviruses, minute virus of mice, Kilham's rat virus, Toolans's H-1 virus of rats)	Subclinical or persistent infection; fetal malformations; hemorrhagic syndrome in rats; Confounding effects on research; hamsters with hamster parvovirus develop periodontal and craniofacial deformities
Rabbit Parvoviruses (Lapine Parvovirus)	Usually no clinical signs; may produce disseminated infection, mild enteritis in young kits

Genus *Erythrovirus*

Virus	Disease
Non-Human Primate Parvoviruses (Simian Parvovirus, Rhesus Parvovirus, Cynomolgus Parvovirus)	Anemia; fetal abnormalities; elated to human B19 virus

Genus *Amdovirus*

Virus	Disease
Aleutian Mink Disease Virus	Adults (primarily mink that are homozygous for Aleutian coat color, which is associated with a Chediak-Higashi type abnormality): Chronic immune complex disease; encephalopathy; Neonates: interstitial pneumonia

Genus *Dependovirus*

Virus	Disease
Goose Parvovirus	Hepatitis; myocarditis; myositis; lethal disease in 8-30 day old goslings
Duck Parvovirus	Hepatitis; myocarditis; myositis in Muscovy ducks

Genus *Bocavirus*

Virus	Disease
Bovine Parvovirus	Rarely associated with clinical disease; mild diarrhea in neonates
Canine Parvovirus 1 (Canine Minute Parvovirus)	Subclinical; rarely causes diarrhea or sudden death in neonates

Tables adapted from *Fenner's Veterinary Virology,* 4th ed. 2011.[3]

Contributing Institution: Yerkes National Primate Research Center
Emory University
954 Gatewood Road
Atlanta, GA 30329
http://www.yerkes.emory.edu/

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CASE III: 07221 (JPC 4017799-00).

Signalment: 6-week-old Holstein-cross bull calf (*Bos taurus*).

History: The calf had chronic ear infections since two weeks of age. No improvement was seen with multiple antibiotics or bilateral myringotomy. Intermittent purulent discharge was seen bilaterally. Persistent worsening neurologic signs include severe ataxia, obtundation, and absence of hind limb reflexes, withdrawal, and deep pain sensation. CT exam of the skull revealed expansion of both tympanic bullae with multifocal areas of marked lysis and soft tissue or caseous material filling the bullae. Similar material was seen in both external ear canals.

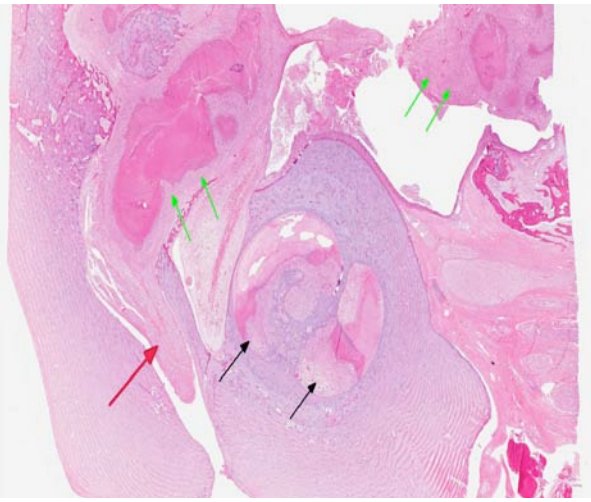
Gross Pathology: Within the external ear canals, tympanic bullae and inner ears is a severe bilateral white purulent to caseous exudate, with osseous thickening of the tympanic bulla. No gross changes to the meninges are seen.

Histopathologic Description: Inner ear: Examined is a cross-section of inner ear consisting primarily of the cochlea within the petrous temporal bone, the adjacent vestibule lined by ciliated epithelium containing abundant mucus cells, and associated vestibulocochlear nerve. In the center of the cochlea is a central core of spongy bone which contains the spiral sensory ganglion. The pseudostratified lining epithelium and associated hair cells of the cochlea are necrotic and replaced by abundant granulation tissue. Filling the vestibule and the cochlea, and abutting portions of the cochlear nerve, is a marked inflammatory exudate comprised of neutrophils, lymphocytes, plasma cells, macrophages associated with severe necrosis, which is evidenced by eosinophilic amorphous cellular debris, basophilic karyorrhectic nuclear debris, and multifocal dystrophic mineralization. Similar necrosis, inflammation and granulation tissue fill the middle ear, which is lined by ciliated pseudostratified epithelium displaying squamous metaplasia. No etiologic agents are seen.

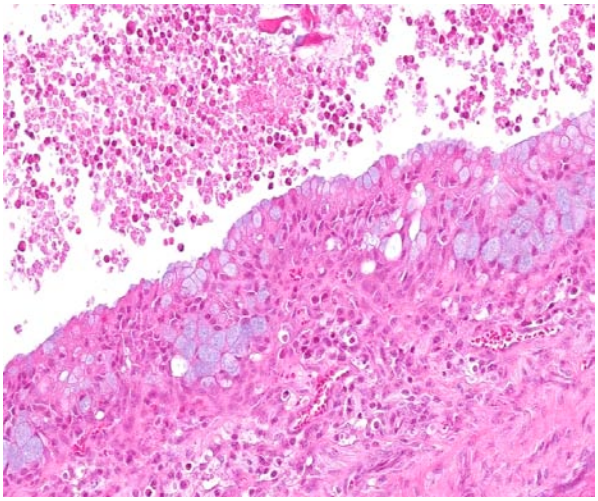
Contributor's Morphologic Diagnosis: Severe, chronic, necrosuppurative otitis interna and media.

Contributor's Comments: Differentials for otitis interna and media in calves includes: *Histophilus somnus, Mannheimia haemolytica, Pasteurella multiocida, Streptococcus* spp., *Actinomyces* spp., *Arcanobacterium pyogenes,* and *Mycoplasma bovis*.[2,5] A heavy growth of *Mycoplasma* was cultured from an ear swab collected at necropsy, with a light growth of both *E. coli* and non-haemolytic *Streptococcus*. Bacteria were not speciated.

Mycoplasma are extracellular bacteria that lack a cell wall and may be transmitted via infected secretions of the respiratory tract, genital tract, or mammary gland from infected cows or other calves.[1] *Mycoplasma* evades the host immune system by suppressing neutrophil and lymphocyte activation, as well as inducing lymphocyte apoptosis.[1] *Mycoplasma* otitis typically affects male dairy calves less than 2 months of age.[3] The pathogenesis of otitis interna and media due to *Mycoplasma* in calves is not completely understood but is thought to involve one or more of the following mechanisms: (1) ingestion of infected milk causing nasopharyngeal infection, with extension through the Eustachian tube to the middle ear, (2)



3-1. Middle and inner ear, calf: The tympanic cavity (green arrows) and vestibular system (black arrows) is lined by a thick layer of granulation tissue with multiple cores of homogenous lytic necrosis. The granulation tissue forms a polypoid mass which occludes the vertical ear canal (red arrows). (HE 4X)



3-2. Middle and inner ear, calf: The normal squamous epithelial lining of the tympanic cavity is lined by pseudostratified ciliated epithelium with numerous goblet cells (metaplastic change). (HE 200X)

immunosuppression or a primary viral infection (BVDV, IBRV) allowing overgrowth of resident flora, (3) direct extension from the external ear canal through the tympanic membrane to the middle and inner ear, or (4) hematogenous spread, which is more commonly seen in rodents, lambs, and swine.[3,5] Infection may then ascend via the vestibulocochlear and facial nerves to the brainstem to cause meningitis. Spleen, liver, kidney, and thymus from this case were tested for bovine viral diarrhea virus via a fluorescent antibody test. Testing for herpesvirus (IBRV) was not performed.

Mycoplasma bovis infections are of economic importance, causing widespread systemic disease, including pneumonia, arthritis, and mastitis.[1,3] Antigenic variation of the surface lipoproteins is thought to contribute to a poor response to antibiotic therapy and evasion of the host immune response.[1,2] Therefore, the cellular and humoral immune responses offer no protection, despite the development of antibody titers.[1] Ingestion of infected milk is thought to be the most likely route of infection, therefore, control measures include pasteurization of colostrum and milk being fed to calves, preventing direct contact between calves, and culling infected cows.[2]

JPC Diagnosis: Middle and inner ear: Otitis media and labrynthitis, necrosuppurative, severe, with osteonecrosis and osteolysis.

Conference Comment: *Mycoplasma* are small, fastidious, pleomorphic, facultative anaeroic bacteria that generally do not replicate outside the host.[4] Although many species are non-pathogenic, several species cause diseases in both humans and animals. *Mycoplasma* species are generally found on the mucosal epithelium of the conjunctiva, nasal cavity, oropharynx, intestinal and genital tracts.

Recently some species have been reclassified from the rickettsial group into the genus *Mycoplasma;* these bacteria are referred to as "hemoplasms," as they have a tropism for red blood cells rather than epithelium.[4]

In addition to the diseases associated with *Mycoplasma bovis* that were adeptly described by the contributor, conference participants also discussed a variety of other *Mycoplasma* species of importance in veterinary medicine[4]:

Conference participants noted the presence of an inflamed mucosal lining, interpreted as a polyp, that

***Mycoplasma* species**	**Hosts**	**Disease**
M. mycoides subsp. *mycoides* (small colony type)	Bovine	Contagious bovine pleuropneumonia
M. bovis	Bovine	Mastitis, pneumonia, arthritis, otitis
M. agalactiae	Ovine, Caprine	Contagious agalactia (mastitis)
M. capricolum subsp. *capripneumoniae*	Caprine	Contagious caprine pleuropneumonia
M. capricolum subsp. *capricolum*	Ovine, Caprine	Septicemia, mastitis, polyarthritis, pneumonia
M. mycoides subsp. *capri* (includes strains previously classified as *M. mycoides mycoides* large colony type)	Ovine, Caprine	Septicemia, pleuropneumonia, mastitis, arthritis
M. ovioneumoniae	Ovine, Caprine	Pneumonia
M. pulmonis	Rodents--rat and mouse	Colonize nasopharynx and middle ear; affect respiratory and reproductive tracts and joints
M. hyopneumoniae	Swine	Enzootic pneumonia
M. hyosynoviae	Swine (10-30 weeks of age)	Polyarthritis
M. hyorhinis	Swine (3-10 weeks of age)	Polyserositis
M. suis	Swine	Mild anemia, poor growth rates
M. ovipneumoniae		mild pneumonia
M. haemofelis	Feline	Feline infectious anemia
M. cynos	Canine	Implicated in kennel cough complex
M. haemocanis	Canine	Mild or subclinical anema; more severe signs in splenectomized animals
M. gallisepticum	Turkeys and Chickens	Chronic respiratory disease; infectious sinusitis
M. synoviae	Turkeys and Chickens	Infectious synovitis
M. meleagridis	Turkeys	Air sacculitis, skeletal abnormalities, reduced hatchability and decreased growth rates
M. felis	Feline, Equine	Conjunctivitis in cats, pleuritis in horses
M. equigenitalium	Equine	Abortion

Table adapted from *Veterinary Microbiology and Microbial Disease.* 2nd ed., 2011[4]

did not appear to be attached to the rest of the tissue. The relationship between the polyp and the other structures was difficult to ascertain in the sections examined. Also, participants noted there is osteolysis of the petrous portion of the temporal bone.

Contributing Institution: Department of Microbiology
Immunology and Pathology
CSU Veterinary Diagnostic Lab
200 West Lake Street
1644 Campus Delivery
Fort Collins, CO 80523-1644
http://www.dlab.colostate.edu/index.htm

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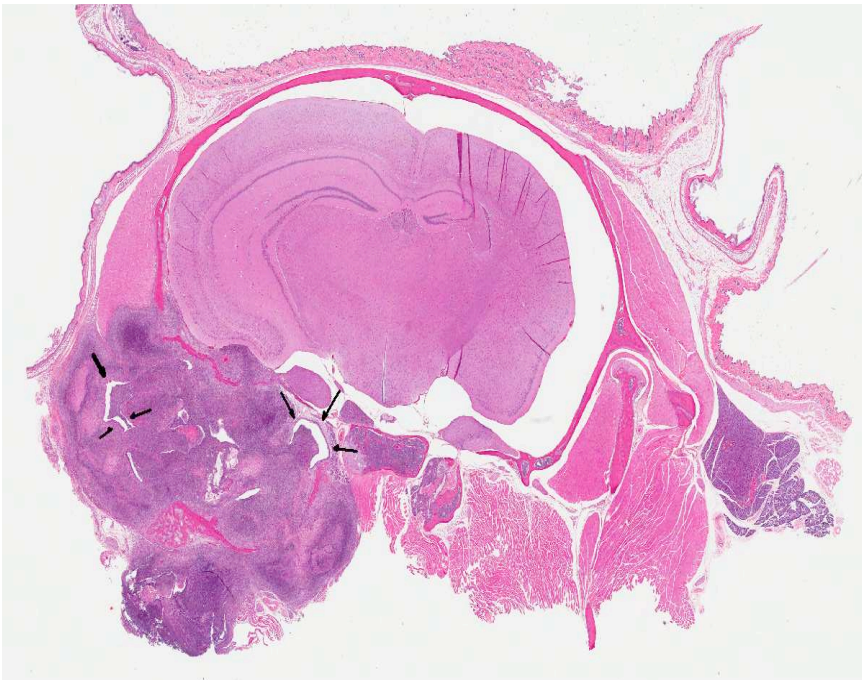
CASE IV: M07-1049 (JPC 3069496).

Signalment: Three six-month-old female SCID-Beige mice, *Mus musculus*.

History: More than six Scid/Beige mice (C.B-lgh-Gbms Tac-Prkdcscid-Ly) from the same colony died during the course of one month. These mice had not been experimentally manipulated and presented weak, lethargic, hunched and losing weight before death. Three sick animals were submitted to the Laboratory of Comparative Pathology for diagnosis. They presented similarly to those that had died previously, except for one animal that had a head tilt to the left.



4-1. Cranium, mouse: The external ear canal (arrows), middle and inner ear is effaced by a focally extensive area of pyogranulomatous inflammation which has resulted in resorption of the cranium and extension, into the cerebrum. (HE 6.0X)

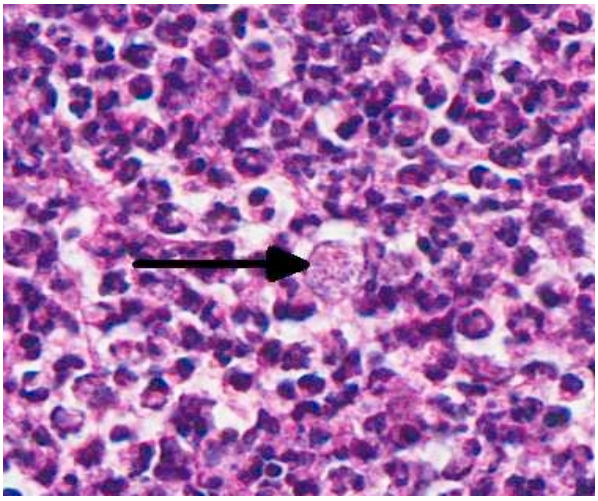
Laboratory Results: Microbiological culture results of the purulent exudate from the abscesses, as well as from blood and spleen indicated *Burkholderia cepacia*. Cultures were sent to the Research Animal Diagnostic Laboratory at the University of Missouri for PCR to differentiate *B. cepacia* from *B. gladioli*. PCR results confirmed *B. cepacia*.

Gross Pathologic Findings: On necropsy, the skin covering the head and neck was removed and one to three, small, rounded, tan to yellow, firm but slightly fluctuant nodules measuring up to 0.5 cm in diameter (abscesses) were found at the base of the ear canal in two out of three mice. The left ear was affected by the largest of these abscesses on the mouse that presented with left head tilt, and the right ear was affected on another mouse. The third mouse did not have gross lesions but all three mice had similar microscopic changes. Purulent material from these abscesses, as well as blood and spleen, were collected for culture. No other gross lesions were detected in the three mice.

Histopathologic Description: Coronal sections of head containing tympanic bulla, surrounding soft tissues and brain are submitted to conference participants. Microscopic appearance varies slightly from slide to slide, according to the depth of the section. In all slides the tympanic bulla is filled and obliterated by a cellular exudate composed of neutrophils, fibrin, proteinaceous and cellular debris, and colonies of small gram negative rods. There is extensive osteolysis of the bulla, with involvement of the surrounding soft tissue and abscess formation within the mandibular musculature locally. A rim of early fibroplasia and large number of rods, free or within macrophages, are noted at the periphery of these abscesses. The inflammation extends into the internal ear in some slides. There is focal lysis of the skull bone adjacent to the tympanic bulla with involvement of the meninges and cerebral parenchyma. The extent of cerebral involvement varies from slide to slide. In areas of cerebral abscess formation the surrounding parenchyma is compressed and a thick band of Gitter cells packed with bacterial rods is noted at the border between necrotic and healthy tissue. There is fibrinoid necrosis of small vessels in the affected cerebral parenchyma. The lesions in all three cases were unilateral. Other changes included miliary histiocytic inflammation of the liver in one of the mice, likely due to septicemia as indicated by the recovery of *B. cepacia* from cultures of blood and spleen.

Contributor's Morphologic Diagnosis:

1. Middle ear: Severe, chronic, suppurative, unilateral otitis media with extensive bulla osteolysis and intralesional gram negative rods.
2. Inner ear (depending on slide): Severe, chronic, suppurative, unilateral otitis interna.
3. Brain and meninges: Severe, chronic, focal, suppurative meningoencephalitis with focal cerebral abscess and intralesional gram negative rods (secondary to otitis).



4-2. Cranium, mouse: The inflammatory infiltrate is composed of numerous degenerate neutrophils and fewer macrophages which occasionally contain 2-3µm bacilli within their cytoplasm (arrow). (HE 400X)

Contributor's Comment: Spontaneous otitis media in immunocompetent mice has been frequently associated with *Mycoplasma pulmonis*, *Pasteurella pneumotropica*, *Pseudomonas aeruginosa*, *Streptococcus* sp., and viral agents such as reovirus.[1] The inflammatory infiltrate can vary from neutrophilic (in most of the bacterial infections) to lymphoplasmacytic and serous and is usually unilateral. When bilateral, the inflammatory infiltrate can differ, i.e. suppurative in one side and serous on the other. Proliferative and papillary lesions, as well as fibrosis of the tympanic cavity are common findings in chronic cases. Causative agents such as bacterial rods or cocci are rarely seen histologically, despite the use of special stains. The incidence of otitis media in mice varies according to strain and age. It is very common in aging 129:B6 mice with a 79 -84% incidence, independent of gender. cBA/J mice are also susceptible to otitis media with a 90% incidence in animals older than one year.[2] However, otitis was found to be more common in aging 129S6 mice than in another CBA strain studied (CBA/Caj).[3]

The most common clinical presentation is head tilt, but neurologic signs such as circling and rolling have been reported in C3H mice and likely depend on the extent and severity of the lesion.[4] A history of previous chemically-induced otitis with ear damage has been found to predispose Rb/3 mice (non-susceptible strain) to audiogenic seizures.[5] In the ICR strain, otitis is manifested as mutilation of the external ear canal due to self-trauma.[6] In addition, Jeff (Jf) mutant mice are also predisposed to otitis, likely due to craniofacial abnormalities in the strain.[7]

In athymic Balb/C-derived nude mice, naturally-occurring Sendai virus infection can cause a chronic respiratory disease characterized by rhinitis, laryngotracheitis, bronchitis/ bronchopneumonia and otitis media (usually suppurative).[8]

The pathogenesis of otitis media in the mouse is uncertain but since it is commonly found without associated otitis externa it may be a sequel of previous viral or bacterial infection of the nasopharynx with ascending infection via the Eustachian tube.[1] Acidification of the drinking water is an effective preventive measure. Tetracycline is recommended for treatment of affected animals.

Otitis media can be experimentally induced in mice by direct injection of the middle ear (often transtympanic) with human pathogenic organisms such as *Streptococcus pneumonia*, *Haemophilus influenzae* or *Moraxella catharralis*.[9] Interleukin-8, as well as *Salmonella typhimurium* endotoxins can also be used to induce otitis media experimentally in mice.[10,11]

In 2004, an outbreak of otitis media associated with *Burkholderia gladioli* was reported in immunosuppressed mice. After an athymic nude mouse presented with head tilt and otitis, several other immunosuppressed mice in the facility presented with similar clinical and pathological findings. Culture of the middle ear of the affected mice initially yielded the phytopathogen *Burkholderia cepacia*, however the isolate was later identified as *Burkholderia gladioli* based on 16S rDNA PCR.[12]

SCID-beige mice are severely immunosuppressed because they lack mature T- and B- lymphocytes ($prkdc^{scid}$ mutation) and have a series of other defects affecting granulocytes, such as the lack of NK cells, reduced bacteriocidal activity of granulocytes, and decreased lysosomal enzymes in neutrophils (Beige or Bg mutation), as noted in the Jackson Laboratory Database. In light of the findings in these mice and the previously reported otitis outbreak in immunosuppressed mice caused by *B.gladioli*, we sent a subculture of the organism to a referral lab at the University of Missouri for PCR. *B. cepacia* was confirmed as the causative agent of otitis in the sick and dying Scid-Beige mice from our colony.

The genus Burkholderia was initially divided in four species: *B. mallei*, *B. pseudomallei*, *B. gladioli* and *B.cepacia*. *B. mallei* is the causative agent of glanders in horses, mules and donkeys; *B. pseudomallei* is the cause of melioidosis, a disease prevalent in Southeast Asia and Australia; *B.gladioli* is a primary plant pathogen but has also been isolated from the sputum of human patients with cystic fibrosis (CF); and *B. cepacia* causes respiratory failure in at least 20% of patients with cystic fibrosis (CF). Currently, the so-called *Burkholderia cepacia*-complex consists of nine

genetically distinct species, all important to humans due to their ability to cause CF-related infections. *B. cepacia* is a small, gram negative, non-motile rod that can be transmitted from individual to individual. When patients with a mild form of cystic fibrosis are infected by *B. cepacia* there is a rapid decline of lung function and resulting respiratory failure with a poor outcome. Immunosuppressed CF patients that received a lung transplant are at high risk of infection with subsequent bacteremia that may result in death (cepacia syndrome).[12,14] In domestic animals, *B. cepacia* has only been reported as a cause of subclinical mastitis in sheep, but may have been reported in the past under the name *Pseudomonas aeruginosa* associated with reptile diseases and infections.[15] In the present cases, large numbers of gram-negative rods can be seen in the histologic sections submitted to conference participants. The culture of *B. cepacia* from blood and spleen confirms a bacteremia and explains the death of the many affected animals. Antibiotic sensitivity results indicated that the *B. cepacia* strain affecting these mice was resistant to some of the most common antibiotics, such as amoxicillin/clavulanic acid, ampicillin, penicillin, tetracycline, oxacillin, and erythromycin. The strain was susceptible to chloramphenicol, ciprofloxacin/ enrofloxacin, gentamicin, trimethoprim/sulfa and amikacin. The remaining colony was treated with Sulfatrim (sulfamethoxazole and trimethoprim) in the feed and recovered from the outbreak.

JPC Diagnosis: Head, sagittal section: Otitis externa, otitis media, labrynthitis, neuritis, myositis, osteomyelitis and meningoencephalitis, necrosuppurative, with intrahistiocytic bacilli.

Conference Comment: The contributor provides a thorough summary of otitis in mice as well a review of *Burkholderia gladioli* and *B.cepacia* infections in both humans and animals. The significant slide variation noted by the contributor was discussed by participants, and the moderator cautioned participants to always evaluate multiple sections when examining the structures of the ear, as a full visualization of the structures of the middle and inner ear requires multiple serial sections to visualize *in toto*. Additionally, in keeping with terminology used in human medicine, the morphologic diagnosis of "labrynthitis" was favored over otitis interna.

Contributing Institution: Memorial Sloan-Kettering Cancer Center
Weill Medical College of Cornell University
Rockefeller University
1275 York Ave.
Box 270; RARC, ZRC-933
New York, NY 10021
http://www.mskcc.orq/ms kcc/html/14131.cfm
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