CASE I: 12-0433 (JPC 4036190).

Signalment: 17-week-old female C57BL/6 mouse, *Mus musculus*.

History: 17-week-old female C57BL/6J mouse which was one of a cohort infected with a sublethal dose of murine cytomegalovirus (MCMV). Six days post-infection, this mouse and one other showed decreased activity; both were culled and submitted for post mortem.

Gross Pathology: The liver and kidneys were pale tan and pale red, respectively. There were multiple petechial and ecchymotic hemorrhages on the surfaces of the gall bladder, small intestine, and the urinary bladder. There were extensive petechial and ecchymotic hemorrhages affecting 30-40% of the total skin area and the paws. Both eyes were diffusely red-black, and there was a small amount of free blood in the pleural cavity.

Laboratory Results: N/A

Histopathologic Description: Liver – Throughout the hepatic parenchyma there are randomly scattered foci of hepatocellular loss, and the tissue is replaced with amorphous eosinophilic material, often with remnant cellular outlines, and with pyknotic/karyorrhectic nuclei (acute necrosis). Associated with these foci, many of the nuclei of hepatocytes and Kupffer cells contain large, often well-demarcated bodies of deeply eosinophilic material with margination and blebbing of chromatin (intranuclear inclusions). Occasionally, small, discrete eosinophilic inclusions are also present in the cytoplasm. In the remaining tissue, the hepatocytes are generally swollen with microvesicular vacuolation (hydropic change).

Spleen – The reticuloendothelial cells of the red pulp are extensively lost and replaced by amorphous eosinophilic material and nuclear debris with little trace of the normal splenic architecture (acute necrosis). The remaining reticuloendothelial cells contain intranuclear inclusions as seen in the liver. The white pulp is relatively preserved, although many lymphocytes in the periarteriolar lymphoid sheaths, individually and in clusters, have shrunken, pyknotic nuclei.

Contributor’s Morphologic Diagnosis:

1. Liver – severe, acute, multifocal to coalescing hepatic necrosis with intranuclear and intracytoplasmic inclusions
2. Spleen –
   a. severe, acute, diffuse red pulp necrosis with intranuclear inclusions
   b. mild, acute, multifocal white pulp lymphoid necrosis
Contributor’s Comment: This mouse was one of a cohort that had been infected with what was described as a sublethal dose of murine cytomegalovirus (MCMV). At day 6 post infection, this mouse and one other from the same cohort were less active than the others in the group, and these 2 were culled. Notwithstanding the description of the administered dose of virus, there were no gross or microscopic findings in either mouse other than those referable to acute MCMV infection.

Murine cytomegalovirus is a DNA virus in the family Herpesviridae, the subfamily Betaherpesvirinae, and the genus Muromegalovirus.\(^4\) The beta herpesviruses are highly host specific viruses that infect humans, mice, rats and guinea pigs (and other mammals, though these are generally less well characterized). MCMV infections in mice have been extensively studied, largely because the course of infection in mice has several pertinent similarities to the course of Human Cytomegalovirus (HCMV) infection, making it a useful model to study the human disease. HCMV is a highly prevalent infection of humans worldwide, with seroprevalence in women of reproductive age varying from approximately 40% (US, much of western Europe, Australia) to >90% (Brazil, Chile, Italy, India, Turkey, Japan).\(^3\) Infection rarely causes clinical signs in immunocompetent individuals. It is, however, a significant cause of morbidity in immunosuppressed individuals and is an important cause of disease (notably of hearing loss and neurological impairment) in children born to mothers who are first infected during pregnancy.\(^5\)

As is typical of herpesviruses, MCMV causes lifelong, persistent infections with intermittent reactivation and virus shedding at times of host immunosuppression. After initial infection, MCMV replication occurs in salivary glands; excretion in saliva appears to be the primary means of transmission through grooming and biting activities. The virus then disseminates and severe infections may induce encephalitis, retinitis, pneumonia, hepatitis, myocarditis, adrenalitis and/or haemopoietic failure. Besides saliva, infective virus is also present in the tears, urine and seminal fluid of infected animals, though the occurrence of natural transmission by means of these secretions has not been well established. Viral DNA replication occurs in the nucleus of infected cells, while production of the capsid protein occurs in the cytoplasm. The capsid proteins are transported to the nucleus where viral packaging takes place; the packaged particles are then transported back to the cytoplasm where they acquire their primary envelope. A second enveloping stage is required before the virions are ready for release. Hence, infection is associated with both intranuclear and intracytoplasmic inclusions. The outcome of MCMV infection is highly dose-dependent – in mouse models of infection, mortality may change from 0% to 100% with only a four-fold increase in dose. Other determinants of susceptibility include strain (BALB/C are highly susceptible; C57BL/6 are resistant) and age of the host (young animals are highly susceptible).

Investigations into the variable susceptibility of mouse strains to MCMV infection has shown that innate immune function, in particular the effectiveness of the natural killer (NK) cell response, is the key determinant of an animal’s capacity to resist the initial infection and to suppress viral replication. A host gene designated Cmv1 has been identified which encodes a natural killer (NK) cell receptor that confers resistance to infection. This receptor, the ligand for which is Ly49H, recognizes MCMV directly and its stimulation induces NK cell expansion and activation.\(^8\) NK cells suppress the infection by direct lysis of infected cells and by producing cytokines to mediate T-cell responses. Counteracting the host immune system are a number of
viral genes, the products of which modify the host immune response by inhibition of NK cell function, blocking of cytotoxic T-lymphocyte function, or inducing resistance to interferon or complement. T-lymphocytes, especially cytotoxic CD8+ cells, are activated by NK cell cytokines, and they play an important role in controlling the MCMV infection and in inducing the state of latency. CD4+ cells are involved to an extent in modulating the CD8+ response, but depletion of these cells does not alter the course of infection. Humoral immunity is relatively unimportant in controlling MCMV infection.

Latent virus in the mouse is identifiable in the salivary glands and in other tissues including lung, spleen, liver, kidney, heart, adrenal glands, and myeloid cells. The severity of reactivated infection may be related to the severity of the initial infection, that is, to the number of cells in which latent virus resides.

**JPC Diagnosis:** 1. Liver: Hepatitis, necrotizing, multifocal to coalescing, severe, with karyomegaly and intranuclear viral inclusions. 2. Spleen: Splenitis, necrotizing, multifocal to coalescing, severe, with lymphocytolysis, karyomegaly and intranuclear viral inclusions.

**Conference Comment:** This is a nice case of this appropriately-named betaherpesvirus, as it demonstrates the marked cytomegaly, karyomegaly and intranuclear inclusions so characteristic to cytomegalovirus infection. Its presentation within a mouse model is relevant to the human form of infection, and these models have aided in the characterization of physiologic mechanisms of immunity as nicely highlighted by the contributor. The degree of necrosis is especially prominent in the spleen in this case, with some conference participants speculating that only extramedullary hematopoietic cells are still viable in most sections.

Natural infections of cytomegalovirus are observed in primates and guinea pigs in addition to mice. Rhesus cytomegalovirus (macacine herpesvirus-3) is the most common opportunistic viral infection in rhesus macaques infected with SIV. It causes multiorgan pathology, with interstitial pneumonia, encephalitis, gastroenteritis, and lymphadenitis being most common. Viral infection is commonly associated with neutrophilic infiltrates, regardless of tissue, and when it occurs in conjunction with simian polyomavirus 40, mesenchymoproliferative enteropathy has been described. Recently, peripheral nerve lesions such as facial neuritis have been identified in macaques, which are likely a bystander effect secondary to inflammation and infected macrophages. In guinea pigs, lesions are largely regarded as incidental findings at necropsy and usually confined to the ductal epithelial cells of the salivary glands.

The contributor details the importance of NK cells in resisting and controlling murine cytomegalovirus (MCMV) infection in mice. Of special interest is the ability of NK cells to clonally expand upon activation of the Ly49H receptor. This stimulated NK cell population persists for months following the resolution of infection, and when the animal is re-introduced to MCMV, a secondary expansion occurs, conferring resistance to re-infection, effectively demonstrating the generation of memory within this cell population. This a well-known feature of T cells in the adaptive immune response, but NK cells are typically described as an important component of the innate immune response, especially concerning virus-infected and neoplastic cells. NK cell regulation occurs through cytokine expression (IL-2 and IL-15 stimulate proliferation, IL-12 activates killing) and inhibitory receptors which recognize the class I MHC molecules expressed by all healthy cells. Viral infection or neoplastic transformation often
reduces class I MHC expression in a cell, thereby activating NK cells to destroy it. Activated NK cells, which all express CD16 and CD56, also secrete IFN-γ which activates macrophages. Interestingly, C57BL/6 (B6) mice are readily recognized as having resistance to MCMV due to Ly49H expression, and its infection in this case indicates a possible deficiency in one of the several factors critical for generation of memory NK cells in MCMV infection: IL-12, microRNA-155, and DNAM-1.

Conference participants discussed their top differentials for necrotizing hepatitis in mice. Other viral infections include: mouse adenovirus-1 (intranuclear inclusions), mouse hepatitis virus (viral syncytia), mousepox (intracytoplasmic inclusions), and mammalian orthoreovirus (only in infant mice). Bacterial causes include Helicobacter spp., Clostridium piliforme, Salmonella spp., Proteus mirabilis, and Listeria monocytogenes as demonstrated in WSC 14-15 Conference 6.

**Contributing Institution:** Murdoch University Veterinary Hospital
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**References:**

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**CASE II:** R05-43 (JPC 3138067)

**Signalment:** Adult female goat.
History: During 2004 and 2005, a goat farm in Northern Taiwan accounted episodes of severe abortion. Two flocks of goats had been introduced from central and Southern Taiwan about half a year prior to the incidence.

Gross Pathologic Findings: At necropsy, the lungs of fetus were mottled, patchy, dark purple to dark grayish red. The placenta was diffusely dark-red with the presence of some yellow to red turbid exudates on the surface of the cotyledonary and intercotyledonary areas. No other gross lesions were found.

Laboratory Results: 1. The detection of *Coxiella burnetii* by PCR with the primer pair of Trans1/Trans2 (687 bp) (Houver et al., 1992) and by nested PCR with the primer pairs of OMP1/OMP2 (501 bp) and OMP3/OMP4 (438 bp) (Zhang GQ et al., 1998) was employed for the final diagnosis of this case.  
2. Other laboratory tests including chlamydiosis by PCR, foot & mouth disease by ELISA, and bluetongue by ELISA were all negative. Bacterial culture of the fetal tissues was also negative.

Histopathologic Description: Microscopically, the placenta had multifocal to locally extensive necrosis in the superficial epithelium in both cotyledons and intercotyledonary areas. The trophoblasts were distended by small, approximately 1µm diameter, basophilic, intracytoplasmic organisms. The underlying stroma had mild to moderate lymphoplasmacytic infiltrates with evident perivascular lymphoplasmacytic aggregates. Macchiavello’s staining revealed heavy intracytoplasmic load of positive microorganisms that were negative for Gram stain. In the fetus, mild to moderate inflammation consisting of lymphocytes, plasma cells, and epithelioid macrophages are frequently seen within in the parenchyma of liver, lungs, and kidney. Occasionally, non-suppurative meningoencephalitis and lymphocytic perivascular cuffing could also be observed.

Contributor’s Morphologic Diagnosis: Placenta, chorioallantoic: Placentitis, necrotizing, acute, multifocal, moderate, with focal vasculitis and intracellular organisms.

Contributor’s Comment: *Coxiella burnetii* is a ubiquitous zoonotic pathogen of Q fever, initially identified in Queensland, Australia, in 1935, after an outbreak of febrile illness among slaughterhouse workers. *Coxiella* was historically considered as a *Rickettsia*, but gene-sequence analysis now classifies it in the order *Legionellales*, family *Coxiellaceae*, genus *Coxiella*. It is an intracellular, small pleomorphic gram-negative bacterium, which completes its life cycle within the phagosomes of infected cells. Although possessing a membrane similar to that of the gram-negative bacteria, it is usually not stained by the Gram technique. According to Raoult et al. (2005), the survival and multiplication of *C. burnetii* in the acidophilic phagosomes prevent antibiotics from killing the bacteria. Increasing pH with lysosomotropic agents such as chloroquine restores the bactericidal activity of doxycycline. The agent has 2 distinct life cycle stages known as the large-cell variant (LCV) and small cell variant. The large-cell variant is the vegetative form of the bacteria seen in infected cells. The small-cell variant (SCV) may be metabolically inactive and is the extracellular and presumably with infectious form of the organism. The SCV form of *C. burnetii* is likely to be long-lived in the environment because of its resistance to osmotic stress, physical disruption, and chemical agents. Two phases of the bacterium have been described: the highly virulent phase I organisms are found in the infected
hosts and insect vectors. The phase II organisms are less virulent or devoid of virulence for mammalian hosts and are obtained through multiple passages of chicken embryos.

Coxiella burnetii is a potential bioterrorism and occupational hazardous agent. Q fever has been described worldwide except in Antarctica and New Zealand. Through the airborne, it can be inhaled by humans. A single organism of C. burnetii may cause disease in a susceptible person. In animals, C. burnetii can infect many animal species, including domestic animals, birds, reptile, wildlife, and arthropods such as ticks. Cats and dogs may represent reservoirs of C. burnetii. Dogs may be infected by tick bites, by consumption of placentas or milk from infected ruminants, and by aerosol. The possibility of human Q fever acquired from infected dogs and cats has been reported. The infected animals are generally asymptomatic, but in mammals they may induce pneumonia, abortion, stillbirth, and delivery of weak lambs, calves or kids. The Coxiella burnetii-infected herds of cows have showed shedding the organisms within the milk for 13 months. The ewe can shed the organisms within the vaginal mucus for 71 days. People who may come into contact with infected animals are at the greatest risk, including farmers, slaughterhouse workers, laboratory workers, and veterinarians. In humans, C. burnetii causes highly variable clinical manifestations, ranging from acute to fatal chronic infections. However, about 60% of the human infections are asymptomatic seroconversions. Acute Q fever in humans displays mainly flu-like symptoms, atypical pneumonia or granulomatous hepatitis. Various rare clinical signs of meningoencephalitis, endocarditis, pericarditis, pancreatitis, and abortion have also been described. It is prudent for pregnant women to limit the contact with infected animals, especially with fetal fluids and unpasteurized milk.

In Taiwan, the first case of acute human C. burnetii infection was reported in 1993. Since 2005, samples have been routinely collected from goats, sheep, cattle, and wildlife for the study of the seroprevalence and histopathological changes of Q fever and the DNA sequence of C. burnetii. The results indicate that Q fever should be considered as a possible pathogen in association with the commonly observed abortion in goats, cattle, and wildlife in Taiwan. To our knowledge, this is the first diagnosis of Coxiella burnetii infection in Taiwan livestock.

**JPC Diagnosis:** Placenta, chorioallantois: Placentitis, necrotizing, subacute, multifocal, severe, with intraepithelial and intratrophoblastic coccobacilli.

**Conference Comment:** The contributor offers an excellent opportunity to identify, describe, and interpret lesions in an organ not often observed in histologic section. Conference participants spent some time discussing the components of the placenta and using the identification of individual layers and their orientation to infer a more specific location of these sections. The placenta is comprised of endometrium (the maternal component) and the fused chorioallantoic membranes (CAM, the fetal component). The indeciduate nature of ruminant placentas implies the maternal and fetal components are in contact but not intimately fused. Additionally, ruminants have cotyledonal placentalation, meaning there are numerous but isolated areas where the CAM contributes to the placenta. In these areas, the CAM villi insert into pockets or crypts in the area of the endometrium known as caruncles. Specific to small ruminants, the caruncles have lost their epithelium, leaving 5 tissue layers which separate maternal and fetal blood: endothelium, connective tissue, and epithelium of the CAM, and endothelium and connective tissue of the endometrium. Conference participants concluded there is no maternal tissue within these sections, and the presence of amnion and the CAM villi indicates these must have been
collected from the cotyledon. Additionally, there are prominent eosinophilic crystals (hemoglobin) within an area of the CAM epithelium indicating the likelihood of a junction between cotyledonary and intercotyledonary placenta, as this is the location where hemophagocytosis is often most prominent.

Although *Coxiella burnetti*’s zoonotic potential earns it the distinction of being an abortion agent of great importance, there are many other infectious causes of abortion outbreaks in ruminants which may induce similar placental lesions. Conference participants discussed other differentials for this case, including bacteria: *Chlamydophila abortus*, *Histophilus somni*, *Yersinia pseudotuberculosis*, *Salmonella* spp., *Trueperella pyogenes*, *Leptospira* spp., *Listeria monocytogenes*, *Campylobacter* spp., and *Brucella* spp.; parasites: *Toxoplasma gondii*, *Neospora caninum*, *Sarcocystis* spp., and *Trichomonas foetus*; and viruses: *Border disease virus*, *Wesselsbron*, *Bluetongue*, *Bovine herpesvirus 1*, and several *Bunyaviruses*. Some are easily differentiated based on lesion distribution in the placenta, such as *T. gondii* and *Y. pseudotuberculosis* which both exclusively target the cotyledons.

*C. burnetti* is known to infect many species and has recently been described as an emerging infectious disease in birds, in which it exhibits variable tissue tropism, with the most recent reports identifying myocarditis and hepatitis in a parrot. Ectoparasites such as ticks are often cited as important vectors of transmission; however, intranasal inoculation was determined to be most effective in causing disease in pregnant goats. Trophoblasts in the allantochorion of the placenta are the primary target of infection and replication of the bacteria; and their main excretion route from these infected goats is during delivery of the aborted fetus, as bacterial DNA was not detected in feces or vaginal mucus in a recent study.

*C. burnetti* is considered highly infectious, though its inoculation does not necessarily translate into reproductive disease. A recent publication identified the organism in 75% of abortion submissions based on PCR, but when combined with histopathology and additional ancillary testing, *C. burnetti* was only determined to be the actual cause in 21% of those cases. The more commonly identified cause of abortions in this study was *T. gondii*, with *Campylobacter* spp. and *Chlamydophila* spp. also being more prevalent than the agent of Q fever.

**Contributing Institution:** Division of Animal Medicine, Animal Technology Institute Taiwan

**References:**


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**CASE III: Case 2 (JPC 4049887)**

**Signalment:** 12-year-old intact male rhesus macaque, *Macaca mulatta.*

**History:** The animal was part of a closed laboratory setting with twenty-five sexually mature rhesus monkeys of different ages and sexes. They were housed in large group cages together in one room with a common air ventilation system. Some of the animals became suspicious with coughing and weight loss. A routine health check was done including a tuberculosis screen. The palpebral skin test of this monkey became positive, indicated by obvious swelling, drooping and erythema of the eyelid within 24 to 48 hours after application. Furthermore, blood testing using the PRIMAGAM®-test, revealed evidence for mycobacterial infection, and chest radiographs showed multiple small-sized shadows throughout the lung. Finally, the presence of mycobacteria within gastric lavage fluid could be demonstrated by microbiological culture and PCR. The animal was humanely euthanized because of animal welfare reasons.

**Gross Pathology:** At necropsy, the animal showed a poor body condition and main pathological findings were located within the respiratory tract. Multiple small firm nodules varying in size from pinpoint to several millimeters in diameter were visible within the lung, affecting mainly the right *lobus cranialis* and *caudalis*. These granulomatous nodules were yellow-white with some of them coalescing to a large necrotic area of 2,5 x 3,5 cm within the right cranial lobe. The tracheobronchial and hilar lymph nodes were moderately enlarged with caseous necrotic centres on cut surface. More than 40 granulomatous nodules of different size were found in the liver and further 15 granulomas were present in the spleen. One single small lesion was detectable within the testis.

**Laboratory Results:** *Mycobacterium tuberculosis* was isolated from aseptically obtained fresh pulmonary and liver tissue by culture and confirmed by PCR. Ziehl-Neelsen stain: negative.

**Histopathologic Description:** Throughout the lung parenchyma, there are multifocal to coalescing caseous granulomas, characterized by a central necrotic area of amorphous
eosinophilic debris surrounded by a small rim of epithelioid macrophages and few multinucleated giant cells with peripherally arranged nuclei (Langhans type). Mineralization of the necrotic centers is not observed. Granulomas are circumscribed by small amounts of fibrous connective tissue, accompanied by various numbers of lymphocytes and plasma cells. In the periphery of larger granulomas, small non-necrotizing granulomas merely composed of epithelioid macrophages and Langhans giant cells with a small outer rim of lymphocytes are present. Some of the larger granulomas break into major airways resulting in cavity formation together with ulceration of the bronchial epithelium and marked infiltration of neutrophilic granulocytes. Expanded bronchial airways contain necrotic debris admixed with several inflammatory cells. Pulmonary lesions were accompanied by varying degrees of alveolar edema and hemorrhage. The lymph node is characterized by moderate to severe follicular hyperplasia with focal solid granuloma formation, composed of epithelioid macrophages, Langhans giant cells and neutrophilic granulocytes.

**Contributor’s Morphologic Diagnosis:** Lung: granulomatous pneumonia, chronic, severe, multifocal to coalescing, with cavity formation and necro-suppurative bronchitis, induced by *Mycobacterium (M.) tuberculosis*, rhesus macaque (*Macaca mulatta*), nonhuman primate. Pulmonary lymph node (not included in all slides): granulomatous lymphadenitis, subacute, moderate, focal, rhesus macaque (*Macaca mulatta*), nonhuman primate.

**Contributor’s Comment:** Tuberculosis in Old World monkeys commonly results in a debilitating pulmonary disease. The most common sign is coughing and weight loss, which should be regarded as an indicator for the disease. The clinical disease state is directly associated with the amount of gross alterations.

The primary lesion of simian tuberculosis is the typical tubercle. Lesions can vary from non-detectable lesions to widely disseminated characteristic granulomas. The granulomas are firm yellow-white or greyish nodules of different size. Palpable firm nodules may affect all major organs, but the lung is the most commonly affected organ system. Gross lesions also include large cavernous and coalescing lesions within the lung and tubercles may extend into the thoracic pleura or trachea. Alterations can be accompanied by enlarged tracheobronchial lymph nodes with focal or multifocal granulomas and loss of nodal architecture to various degrees. Advanced stages of the disease are characterized by secondary spread to spleen, kidney, liver and different lymph nodes. Dissemination to spleen, liver, and other organs varies among monkeys and does not necessarily correlate with the severity of lung involvement.

Microscopic findings are dependent on duration and extension of the disease. The histopathological hallmark is the granuloma. In experimental studies, a wide spectrum of different granuloma types can be seen depending on the stage of disease. Early stages of the disease are characterized by small granulomas consisting of circumscribed accumulations of epithelioid cells and few Langhans-type giant cells confined to the lung or the intestinal tract. Advanced stages of the disease are characterized by the classic tubercle formation. Tubercles are typical caseous granulomas of varying size containing a caseous center consisting of acellular necrotic debris or proteinaceous material. The central cores are surrounded by a mantle zone of epithelioid cells and a band of plasmacytic and lymphocytic cells interspersed with only a few Langhans-type giant cells. In contrast to other animal species, a fibrous capsule is usually not
found in nonhuman primates. Non-necrotizing granulomas are merely composed of epithelioid macrophages and Langhans-giant cells surrounded by lymphocytes. These solid granulomas exclusively occur in active TB. Fibrocalcific granulomas are composed of various combinations of fibrous connective tissue and mineral deposition. These lesions are generally observed in monkeys with latent infection. However, calcification is usually rare or lacking. Experimental studies show that only long-term non-progressive lesions tend to calcify. The number of acid-fast bacilli within granulomatous lesions can vary considerably. Hence, it can be difficult to demonstrate the bacteria within histologic slides, like in the present cases. Therefore, the microscopic examination alone is not sufficient for the diagnosis of simian tuberculosis. A combination of different test methods based on serology, x-ray, histology and microbiology is advisable to accurately diagnose simian TB.

**JPC Diagnosis:** Lung: Pyogranulomas, multifocal to coalescing, numerous.

**Conference Comment:** This is a classic case of the primate tuberculosis, in which the textbook pyogranulomas seem to entirely efface nearly every bronchiole. Not every slide includes the described lymph node, but of those which do, some have only lymphoid hyperplasia while others have a more prominent granulomatous infiltrate.

Along with the contributor, we also performed acid-fast stains on these sections and did not identify a single organism. This is often typical of tuberculoid granulomas, which are T\(_H\)1-driven lymphocytic responses to infection of *Mycobacterium* spp. The macrophages in these lesions are actually utilized by the bacteria to facilitate its spread, as their cellular uptake is promoted by the recognition of multiple membrane pathogen receptors including complement, mannose, surfactant protein, and CD14. Within the macrophage, they disrupt phagosome-lysosome fusion and are able to grow, replicate, and subsequently disseminate.

Following this period of bacterial proliferation, typically about 3 weeks after infection in humans, T\(_H\)1 lymphocytes are produced following stimulation of TLR2 by mycobacterial ligands which ramp up production of IL-12. T\(_H\)1 cells produce IFN-\(\gamma\) in abundance, which are the critical mediators enabling macrophages to contain the infection. Additionally, IFN-\(\gamma\) orchestrates the formation of granulomas by inducing differentiation of macrophages into their epithelioid versions which aggregate and often fuse to form giant cells. The immune response, while effective as evidenced by the lack of bacteria present in the current case, comes at the cost of tissue destruction.

Interestingly, a von Kossa stain demonstrated no mineral within the granulomas in this case. Central mineralization occurs commonly in stage III of granuloma formation (which occurs several weeks to a month following infection) and is characterized by a mineralized necrotic center with more organized zones of lymphocytes, fibroblasts and a fibrous connective tissue capsule. Mineralization is not usually present in active infections. The lesions in this case also lack prominent fibrous capsules and the abundant neutrophils present with lack of mineralization suggest an earlier form of disease such as stage I or II. As the contributor mentioned, this presentation may be more common in primates, as mineralization is less often described in contrast to its common occurrence in cattle.
**Contributing Institution:**  German Primate Center

http://dpz.eu

**References:**

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**CASE IV:** 09A029 (JPC 3134289)

**Signalment:** 1.6 yr male Indian rhesus monkey, *Macaca mulatta.*

**History:** This animal was moved indoors from an outdoor corral one week prior to developing a head tilt, tremors, and anorexia overnight. It failed to respond to therapy.

**Gross Pathologic Findings:** Meninges were congested and cloudy. A 1 cm area of hemorrhage and malacia was observed in the right frontal lobe of the brain. Smaller petechial hemorrhages were noted bilaterally throughout the parenchyma.

**Laboratory Results:** CBC: 5.67 Rbc, 12.6 Hgb, 38.3 Hct, 20.1 WBC, 84.2 Seg, 0.5 Eo, 0.2 Eo, 0.2 Bso, 2.8 Mno, 12.3 Lym, 403000 Plt, 0 Rtc

Chem: 145 Na, 104 Cl, 6.8 Pro, 4 Alb, 2.8 Glob, 1.4 A/G, 9.9 Ca, 26 BUN, 83 Glu, 0.56 Crt, 4.1 Ph

Brain Culture: Alpha-hemolytic *streptococcus*
**Histopathologic Description:** The macroscopic cerebral hemorrhage represents a large zone of malacia filled with fibrin, hemorrhage, necrotic cellular debris and infiltrated with foamy histiocytes, lymphocytes, plasma cells and neutrophils. Some vessels contain fibrin thrombi and their walls are disrupted by eosinophilic fibrinoid material and degenerating neutrophils. Many vessels in the meninges and parenchyma are lined by plump endothelial cells and are cuffed by mixed leukocytes. Neutrophils predominate in and around vessels with necrotic walls. Adjacent neuropil is vacuolated and contains swollen degenerated neuron cell bodies and axonal degeneration (spheroid material).

**Contributor’s Morphologic Diagnosis:** Subacute, severe necrohemorrhagic encephalomalacia with meningitis and vasculitis, alpha-hemolytic streptococcus.

**Contributor’s Comment:** The most common human CNS infections are bacterial, although the incidence in the US (6 per 100,000) is considerably lower than in developing countries (300-400 per 100,000).³ Vaccination has reduced the importance of *Haemophilus influenzae type b* but *Neisseria meningitides* and *Streptococcus pneumoniae* are still leading causes.⁴ In our colony, the incident rate is more comparable to an unvaccinated population (82 per 100,000) with *S. pneumoniae*, the most common pathogen, while *Neisseria* and *Haemophilus* are not detected. *Alpha streptococcus* is a less frequent but equally devastating pathogen in young rhesus monkeys. *Alpha streptococcus*, sometimes called viridans streptococcus, can be differentiated from *S. pneumoniae* by resistance to the optochin disk, lack of bile solubility, and failure to grow on 6.5% NaCL. Although the viridans group is not known for factors that facilitate tissue invasion, the ability to bind to laminin may confer pathogenic ability to a select few in the normal flora of mucosal surfaces.⁷ Predisposing conditions such as diabetes, cirrhosis, cancer and chronic sinusitis were not detected in this animal.

**JPC Diagnosis:** Brain, cerebrum: Encephalitis, necrotizing and hemorrhagic, multifocal, acute, severe.

**Conference Comment:** Conference participants enjoyed describing and discussing this case, although they could not arrive at a consensus on a pathogenesis of the vascular lesions. Most attributed a majority of the pathology to a primary infarct, as a pale central area surrounding an infarcted vessel is distinctly void of any neutrophils. Immediately surrounding the pale area are abundant neutrophils suggesting a blockage of the arterial supply prevented leukocyte migration to the necrotic center. The additional vascular lesions, observed occasionally as fibrinoid change within vessels or necrotizing vasculitis, may be due to toxin secretion from the cultured gram-positive bacteria or a more chronic hypersensitivity reaction. Regardless, necrotizing vasculitis and fibrin thrombi with subsequent ischemic necrosis does routinely occur in primates with streptococcal meningitis.²

*Streptococcus* spp. are gram-positive coccic that grow in pairs or long chains. There are over 50 recognized species, and all are divided into one of three groups based on their hemolytic properties.⁷ The α-hemolytic group is comprised of *Streptococcus pneumoniae*, a common cause of pneumonia in adult people and meningitis in children, and some members of the viridans group.⁴ The viridians group is largely composed of commensal bacteria, especially of the oral cavity. Some members of this group are nonhemolytic, thus not classified with the rest of the α-hemolytic group; however, characteristic to all viridians streptococci is their lack of Lancefield antigens. The β-hemolytic group all express Lancefield antigens and are typed accordingly.⁷
Streptococcus pneumoniae has been one of the most extensively studied of all the microorganisms, lending credit to its persistence in the crowded field of important pathogens. The identification of DNA was inferred from work performed on this bacterium, during which a “transforming principle” was described by the discovery that nonencapsulated strains could be converted into capsulated strains in 1928. Other important discoveries derived from this pathogen include the efficacy of penicillin, the demonstration of antibody formation to polysaccharides, and the identification of regulatory T-lymphocytes.

More recently, the two-component system (TCS) has been characterized as a key mechanism through which bacteria perceive and respond to their environment. The TCS model is composed of two proteins: a membrane-associated sensor histidine kinase (HK) and a cytoplasmic cognate response regulator (RR). The HK receives external stimuli and phosphorylates the RR which regulates gene expression or protein function. The importance of the TCS is not only in bacterial pathogenicity, but also in osmoregulation, chemotaxis, sporulation, and photosynthesis. These systems are absent from vertebrates, and thus have received attention as potential targets for antimicrobials. Many specific TSCs have been identified in Streptococcus spp., and whether they lead to the generation of a new class of antimicrobials remains to be seen.

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**References:**