CASE 1: L13-16980 (JPC 4048155).

Signalment: 3-year-old female rat, Rattus norvegicus.

History: This pet rat presented for less than 12 hours of neurologic signs and urinary incontinence. On examination the rat had left head tilt with bilateral mydriasis and absent pupillary light reflexes. The body was also tilted to the left. Ventral to the right ear, there was a hyperemic swelling; when compressed, pus exuded from the ear canal. Over the next 12 hours the animal was treated with antibiotics but deteriorated and was euthanized.

Gross Pathology: There was a 1 x 1 x 0.3 cm swelling immediately ventral to the right pinna. On manipulation of the swelling, yellow, creamy exudate mixed with heterogeneous, firm, yellow and brown material oozed from the ear canal. The right tympanic bulla was enlarged and measured 10 x 9 x 4 mm, whereas the left tympanic bulla measured 7 x 3 x 3 mm. The right cerebral hemisphere was slightly swollen and there was thick, creamy, yellow-green, opaque exudate in the leptomeninges covering the rostral cerebellum.

Laboratory Results: Postmortem cultures yielded Prevotella melaninogenica from the ear, brain, and an acutely congested and edematous lung. Enterococcus sp. was also isolated from the ear. Mycoplasma culture on the same set of samples was negative.

Histopathologic Description: Skull (multiple levels): Bilaterally associated with ulceration of the external ear canal, extending into and multifocally effacing the tympanic cavities of the middle ears, and extending into the cranium on the right side, are innumerable degenerate neutrophils and fewer macrophages mixed with abundant cellular debris, edema, and fibrin (lytic necrosis). In the right external ear, there is fibropapillomatous epithelial proliferation, with abundant underlying granulation tissue that entraps squamous epithelial cells and debris. The right tympanic bulla is four times normal size and contains large amounts of keratin and multifocal inflammatory cell infiltrate as previously described. The cell infiltrate also fills the left tympanic cavity and bilaterally infiltrates the extensively ulcerated stratified squamous epithelium and the subepithelial tissue of the middle ears, accompanied by multifocal resorption and remodeling of the temporal bones. There is no evidence of the auditory ossicles on
either side. Within the submucosal stroma of the middle ears, the infiltrate is predominantly lymphoplasmacytic. There is also goblet cell metaplasia/hyperplasia and multiple glands distended with amphophilic (mucous) secretion. The inflammation extends along and multifocally infiltrates degenerate trigeminal nerves, and results in necrosis of approximately 10% of the right cerebral hemisphere. Adjacent to the necrotic regions in the brain, multifocal areas of neuropil are rarefied (malacia). Multiple neurons are degenerate, with regional gliosis. Within the right lateral and the third ventricles of the brain, there are small numbers of neutrophils, macrophages, lymphocytes, and plasma cells. The midline of the cerebrum is slightly displaced to the left. Multifocally and primarily at the edges of the foci of necrosis in the tympanic cavities and in the brain there are colonies of gram-negative rod-shaped and gram-positive coccoid bacteria. The inflammatory process also involves the laryngeal submucosa and skeletal muscle, resulting in myofiber necrosis characterized by hyalinization and fragmentation. The intracranial bone marrow appears hypercellular, with moderate myeloid hyperplasia.

**Contributor’s Morphologic Diagnosis:** Ears: Otitis externa and media, bilateral, proliferative, ulcerative, and necrosuppurative, chronic, severe, with intralesional mixed bacteria and extension to encephalitis.

**Contributor’s Comment:** Otitis media (OM) is common in laboratory and pet rats. An otoscopic study of 80 Wistar laboratory rats housed in barrier versus non-barrier units showed incidences of spontaneous OM to be 5% and 20%, respectively. The condition is likely underestimated in most laboratory and domestic mammals, with the notable exception of horses, in which OM is usually only associated with temporohyoid osteoarthropathy. In developed countries, 80% of children will have an episode of otitis media by their third birthday, and 40% will have at least six episodes by age seven. Primary or secondary bacterial infection is virtually ubiquitous in otitis media, and bacterial ascension through the tympanic bulla or via the Eustachian tube are proven routes of entry. Evidence for hematogenous infection remains circumstantial. The progressive pathology of otitis media is well-described. To reflect the clinical progression of OM, otolaryngology provides an instructive classification scheme: 1) Otitis Media with Effusion (OME) corresponds to asymptomatic persistent middle ear effusion, 2) Acute Otitis Media (AOM) is recurrent middle ear effusion with clinical signs, and 3) Chronic Suppurative Otitis Media (CSOM) refers to discharge through a perforated tympanic membrane for greater than 2 weeks. Complications of OM include hearing impairment, vestibulopathy, extension to otitis interna, and meningoencephalitis. Globally
21,000 people die annually due to OM-related complications. The presence of stratified squamous epithelium, goblet cell metaplasia/hyperplasia and mucus-producing submucosal glands in the middle ear is consistent with a chronic suppurative otitis media in this rat. Although not evident in the current case, concurrent otitis interna was strongly suspected due to extension of the inflammatory process into the brain.

Clinical signs of OM are ear pain, odor, head tilt, vestibular signs, scratching at the ear, and neurologic signs related to meningoencephalitis. Discharge/debris from and below the ear canal may be seen. Otoscopic examination may reveal an inflamed tympanic membrane, pus behind the membrane, or membrane rupture. Clinical diagnosis can be aided by radiography, CT scan, cytology, and bacteriology.

Organisms commonly implicated in otitis media in rats are *Mycoplasma pulmonis*, *Streptococcus pneumonia*, *Pasteurella pneumotropica*, *Staphylococcus* sp., *Corynebacterium kutscheri*, and *Klebsiella* sp. In this case *Mycoplasma* culture of the ear, brain, and lung was negative.

*Prevotella melaninogenica* (formerly *Bacteroides melaninogenicus*) is an anaerobic gram-negative bacillus. Of the anaerobic gram-negative bacilli involved in animal diseases, *Fusobacterium* is the most important genus, and other genera include Bacteroides, *Dichelobacter*, and *Porphyromonas*. *P. melaninogenica* is also classified as a “black pigmented bacterium” due to its production of black iron metabolites on blood-containing media; thus, the species name is a misnomer as melanin pigments are tyrosine-based. Virulence factors include hemolysin, neuraminidase, and collagenase. *P. melaninogenica* is also classified as a “black pigmented bacterium” due to its production of black iron metabolites on blood-containing media; thus, the species name is a misnomer as melanin pigments are tyrosine-based. Virulence factors include hemolysin, neuraminidase, and collagenase. A search of the current veterinary literature identified *P. melaninogenica* as a common pathogen in bovine footrot and bite wounds from dogs and cats, but no reports of otitis in rats were found.

**JPC Diagnosis:**

2. Middle ear: Cholesteatoma.
3. Middle ear: Otitis media, suppurative, focally extensive, with mucosal ulceration, squamous metaplasia, and bone remodeling.

**Conference Comment:** The opportunity to observe an entire pathologic process in a single
section makes this a truly unique case. It is likely the initial insult was bacterial colonization within the nasopharynx that spread up the Eustachian tube to the middle ear followed by its extension into the calvarium. Of special importance here is the formation of a cholesteatoma within the middle ear. Cholesteatomas are common in people in this geographic location, usually associated with chronic otitis media. They are non-neoplastic, cystic lesions lined by keratinizing squamous epithelium or metaplastic mucous-secreting epithelium and filled with amorphous debris. The pathogenesis behind their formation is still unclear, but a widely acknowledged theory is that the negative pressure and dysfunction of the Eustachian tube causes a deepening retraction pocket that, when obstructed, desquamated keratin cannot be cleared from the recess. They are known to erode the ossicles, labyrinth, and adjacent bone by their production of cytokines such as RANKL and MMP’s, and it is likely this specific lesion permitted invasion of the calvarium in this case.

With the common occurrence of ear infections in infants and children, there is an extensive amount of published information regarding their pathogenesis and treatment in people, most of which revolves around the formation of biofilms. Biofilms are complex bacterial communities that adhere to the surface of implanted biomaterial or mucosa and play a major role in chronic ear infections. In addition to enhancing adherence, biofilms increase bacterial virulence by protecting the microbes from immune effector mechanisms and increases their resistance to antimicrobial drugs. In the ear, biofilms may contribute to cholesteatoma formation, as well as suppurative and non-suppurative otitis, and their formation can exacerbate the infection. This is why the first choice of treatment with internal ear infections in people is often surgery, and why all tissue with potential to harbor biofilms must be removed at that time or risk recurrence of infection.

Conference participants were afforded the opportunity to review the intricate and minute anatomy of the outer, middle and inner ear, during which no less than thirty specific anatomical terms were described within the cochlea alone. Nomenclature aside, appropriate function of the ear requires vibration of the tympanic membrane by sound waves, which are then conducted into the cochlea via the ossicles of the middle ear (malleus, incus and stapes) by a push on the oval window. This movement incites a fluid wave within the cochlea, which is completely full of lymph fluid called endolymph. Where along the snail-shaped cochlea this wave intersects with the Organ of Corti and subsequently delivers information to the brain depends on its frequency. Of vital importance to this conduction system is the maintenance of a strong positive endocochlear potential within the endolymph, which requires precise control and recycling of potassium. In fact, potassium recycling defects in the cochlea is an important cause of deafness in people.

**Contributing Institution:** Department of Pathobiological Sciences
School of Veterinary Medicine
Louisiana State University
http://www1.vetmed.lsu.edu/PBS/index.html
Louisiana Animal Disease Diagnostic Laboratory
http://www1.vetmed.lsu.edu/laddl/index.html

**References:**
2. Chen J, Zhao HB. The role of an inwardly rectifying K(+) channel (Kir4.1) in the inner ear and hearing loss. *Neuroscience*. 2014;265:137-146.
CASE II: E 1061/14 (JPC 4048856).

**Signalment:** Adult female B6 mouse, *Mus musculus*.

**History:** The mouse was mated and suspected to be pregnant. About 14 days later the keepers suggested that the animal was not pregnant, but an abdominal swelling was reported. The animal showed a good general constitution, and food and water intake were unremarkable. The abdominal swelling was indolent. The animal was euthanized and a necropsy was performed. Formalin-fixed samples of the abdominal wall, ovaries, liver, colon and spleen were submitted for microscopic investigation.

**Gross Pathology:** The spleen was moderately enlarged and multiple, whitish nodules measuring up to 2 mm in diameter were observed. The liver showed a light brown coloration and was extensively and firmly attached to the small and large intestines as well as to abdominal fatty tissue. The ovaries and abdominal wall could not be identified grossly with certainty.

**Laboratory Results:** None.

**Histopathologic Description:** The slide shows a section of an abdominal mass of approximately 0.75 cm in diameter with adjacent skeletal muscle of the abdominal wall and abdominal fatty tissue. The mass is surrounded by a thick capsule consisting of fibroblasts and large amounts of collagenous fibers. In the center large areas of amorphous acellular eosinophilic material is present (necrosis). Additionally, there are segments of necrotic skin with keratin lamellae and necrotic hair follicles characterized by round to oval circles with central accumulation of dark brown coarse granular pigment. Furthermore, necrotic skeletal muscles are identifiable as eosinophilic straps with cross-striations. Islands of large pale basophilic cells arranged in a honeycomb-like pattern measuring up to 40 µm in diameter with pale eosinophilic predominantly centrally located shadows of nuclei (necrotic cartilage) are present. Adjacent to the necrotic cartilage, there are partly mineralized areas of necrotic bone tissue consisting of thin cortical structures and trabeculae without identifiable cellular elements. At the periphery of the mass, there is a marked accumulation of cellular debris mostly consisting of degenerate neutrophils with numerous viable neutrophils and as well as foamy macrophages. Within and around the fibrous capsule, a mild to moderate infiltration of plasma cells and lymphocytes is found. The serosa, abdominal striated muscle, and fatty tissue show a multifocal to coalescing moderate infiltration of lymphocytes and plasma cells. Adjacent to the mass parts of the exocrine pancreas are present (not in all sections), which is similarly infiltrated as described above.

**Contributor's Morphologic Diagnosis:** Abdominal cavity, Serositis, severe, pyogranulomatous, chronic with necrotic skin, musculature, cartilage and bone; findings consistent with abdominal pregnancy.

Myositis, pancreatitis, steatitis, multifocal to coalescing, moderate, lymphoplasmacytic, chronic.

**Contributor's Comment:** Abdominal pregnancy is a form of ectopic or extrauterine pregnancy that is characterized by an abdominal location of the embryo or fetus. Especially in early stages of gestation, the zygote has the ability to adhere to several maternal tissues and to connect to
maternal blood vessels. Due to limitations of space and nutritional resources, embryonic or fetal death occurs in some cases concurrent with the observation of clinical signs of the mother. A distinction is made between primary and secondary ectopic pregnancy. In cases of primary ectopic pregnancy the zygote directly adheres to maternal tissue other than the uterus. Primary ectopic pregnancies in humans can be categorized into three subgroups.

Ovarian pregnancy (graviditas ovarica): In these cases, the embryo develops in direct contact with the ovary. Only human cases have been reported so far.

Tube pregnancy (graviditas tubaria): It is the most frequently occurring primary ectopic pregnancy in humans that is often associated with severe intraabdominal hemorrhages. No cases have been reported in animals other than non-human primates.

Abdominal pregnancy (graviditas abdominalis): A true primary abdominal pregnancy has not been described in animals. One reason might be the fact that due to gastrointestinal movements in animals, the implantation of the zygote is inhibited. In contrast, due to the upright body position in humans, the zygote has a better chance to connect with extra-uterine maternal tissue caused by the relatively small pelvic cavity and the lesser influence of gastrointestinal movements in this area.

In secondary abdominal pregnancy the embryo or fetus starts to develop within the uterus. Subsequently, it is dislocated to the abdominal cavity and attached to extra-uterine maternal tissue with connection to maternal blood vessels. Secondary abdominal pregnancies are reported in all domestic animals with a declining frequency: cattle, rabbit, sheep, dog, pig, cat, goat and horse. In mice, abdominal pregnancy has also been induced experimentally with living fetuses placed into the abdominal cavity.

Secondary abdominal pregnancy is often caused by trauma or spontaneous uterine rupture. This process is called internal birth (partus internus). In most cases, when the abdominal pregnancy is recognized the cause of the uterine rupture cannot be identified anymore. Spontaneous uterine ruptures are often a consequence of uterine torsions or other pathological uterine conditions. Directly after dislocation of the embryo or fetus into the abdominal cavity the uterus contracts and uterine contractions stop immediately. The fate of the abdominal embryo or fetus depends on the ability to connect to maternal tissue and to ensure the connection to maternal blood vessels to guarantee nutritional supply. In cases with intact amniotic membranes the probability for an embryonic or fetal survival increases.

In the maternal abdominal cavity, the embryo or fetus is initially recognized as a foreign body and within hours a circumscribed, serofibrinous inflammation starts which develops to an adhesive peritoneal reaction without involvement.
of infectious agents. Often a capsule is formed. In cases with neoplacentation, the placental fragments lose their species-specific properties and transform into irregularly formed islets of diffuse placental connections. Neoplacentations can occur in various abdominal organs. Neoplacentations within the mesentery or the great omentum normally do not cause clinical signs of the mother. Neoplacentations in other organs may be associated with severe clinical signs of the mother due to disturbances of normal organ function.

During the whole duration of abdominal pregnancy the embryo or fetus can die due to nutritional failure. If this happens the embryo or fetus will undergo mummification or maceration and may induce a reactive inflammatory reaction. This may lead to abscess formation and fistulation through the abdominal wall or inflammatory involvement of several abdominal organs.²

**JPC Diagnosis:** Uterus, abdominal cavity: Metritis and peritonitis, pyogranulomatous, focal, encapsulated, with myositis and a macerated fetus.

**Conference Comment:** This is an interesting and descriptively challenging case during which many participants debated whether the fetus was within the uterus or free in the abdominal cavity. Some sections contained a small piece of epithelium that to most looked like gestational uterine epithelial cells. Immunohistochemical staining of the section for smooth muscle actin demonstrated that smooth muscle was present surrounding much of the lesion. The combined findings left many to conclude the pregnancy began in the uterus that subsequently ruptured and adhered to the peritoneal wall. The contributor provides an excellent overview of ectopic pregnancies, of which only secondary abdominal pregnancy has been reported in animals, which supports the opinion of most participants.

Another important question pertaining to this case is regarding the age of the fetus. Many deliberated
as to its gestational age, and subsequently, how long prior to necropsy it had died. Ectopic pregnancies occur most commonly in the fallopian tube in people, and fetal development occurs as usual until, in some instances, its size outgrows the tissue's ability to expand. This may cause a rupture 6-8 weeks into gestation of a fallopian tube pregnancy. With an average gestation of just 20 days in mice, the history of mating 14 days prior to necropsy indicates this fetus may have been close to term. Whether the majority of this development occurred within the uterus prior to a rupture or largely took place while attached to the abdominal wall as observed in these slides remains a point of speculation.

**Contributing Institution:** Department of Pathology, University of Veterinary Medicine Hannover, Germany
http://www.tiho-hannover.de/kliniken-institute/institute/institut-fuer-pathologie/

**References:**
CASE III: CP 8322 (JPC 4049001).

Signalment: 10-month-old female BALB/c, *Mus musculus*.

History: The mouse was group housed with other females in an individually ventilated cage (IVC) system. No experimental procedures had been performed on the mouse as she was part of a cohort being allowed to age prior to study commencement. The technicians noted a mass on the side of the face/neck, culled the animal and removed tissues for histological assessment.

Gross Pathology: A piece of haired skin with a 10 mm diameter cavitated, fluid filled mass was received in formalin. The mass had a multifocally thin wall (less than 1 mm fixed tissue thickness) but in other foci the wall of the cavitation was expanded by beige homogeneous tissue up to 5 mm in thickness. The fluid contained within the cavitated structure was clear. Thoracic and abdominal viscera were also submitted fixed in formalin and no other abnormalities were detected.

Laboratory Results: None.

Histopathologic Description: Within the subcutis of the haired skin, adjacent to the salivary tissue and lymph node, is an unencapsulated, but well-circumscribed, neoplastic mass, which is bluntly infiltrative in some tissue planes, and moderately densely cellular. The neoplastic cells are arranged in heterogeneous patterns including poorly defined clusters, occasionally in tubular structures, and also multifocally in sheets and broad bundles and streams, supported by a multifocally dense collagenous stroma. The cells are arranged around variably sized, but frequently large, cavitations and in some foci the cells palisade around the spaces. The neoplastic cells are pleomorphic polygonal to spindle-shaped, and are moderately sized, with indistinct cell margins and a moderate amount of eosinophilic cytoplasm. The nuclei are frequently oval and exhibit lightly stippled to clumped chromatin and a variable number of variably prominent nucleoli. The mitotic rate is high at 35 mitoses per 10 high power fields. There are foci of necrosis characterized by nuclear karyorrhexis and accumulations of amorphous eosinophilic material. Multifocal aggregates of small to moderate numbers of lymphocytes, with lesser numbers of plasma cells, are present multifocally. The cavitations within the mass multifocally contain strands of pale eosinophilic material and there are multifocal groupings of small numbers of macrophages, some of which contain grey to amphophilic intracytoplasmic material.

Contributor’s Morphologic Diagnosis: Haired skin on side of face/neck: Myoepithelial carcinoma.

Contributor’s Comment: In the planes of tissue examined, the lesion is consistent with a myoepithelial carcinoma arising from the adjacent salivary tissue (not present on all sections).
Alternative differential diagnoses based on the site and gross morphology included a Harderian gland cystadenoma, but the histological appearance of the specimen was most consistent with a tumor arising from the myoepithelial cells of the salivary gland. Myoepitheliomas generally exhibit a low mitotic rate, but this example exhibited frequent mitoses and thus classification as a myoepithelial carcinoma was preferred. The cavitations exhibited by this mass are considered characteristic and are frequently the result of necrosis leading to the formation of “cyst-like” structures. Had immunohistochemical staining been undertaken, myoepitheliomas stain positively for keratins 5 and 14, reflecting their myoepithelial origin.

Although myoepithelial tumors are infrequent in most strains of mice, they are relatively more common in BALB/c mice, especially females, such as in the present case. They most frequently arise from the submaxillary and parotid salivary glands and present as swellings of the subcutaneous tissue of the ventral neck. A retrospective study of 142 myoepitheliomas determined that these tumors also occur spontaneously in several other inbred strains of laboratory mice including A/HeJ, A/J, LLC.A/Ckc and NOD/Lt. Recently, salivary carcinomas exhibiting myoepithelial and basal cell differentiation have been reported in the Justy mutant mouse strain (background: C3HeB/FeJ) which bears a recessive mutation in the Gon4l gene that regulates gene expression during development.

Myoepithelial carcinomas are rare in humans and in the domestic species although there are at least two reports of myoepithelial carcinoma (synonym: malignant myoepithelioma) in dogs.

**JPC Diagnosis:** Parotid gland: Myoepithelioma.

**Conference Comment:** This is a rarely reported neoplasm in domestic species (other than the mouse), and without distinguishing features, its specific histogenesis is often left to immunohistochemistry. In this particular case, we ran pancytokeratin, vimentin and smooth muscle actin, with only pancytokeratin yielding positive staining. Myoepithelial cells are modified epithelial cells with contractile properties. They exist in glandular tissue, such as salivary and mammary glands, and function by forcing secretions into and through a duct system with their contraction. Myoepithelium stains positive for both epithelial and muscle markers; however, their staining seems to be more variable compared to the apparent high specificity of CK5 and CK14 as mentioned by the contributor.

The question of malignancy was debated in this case, as some pointed out the well-circumscribed appearance and uniform cell population. We agree with the contributor, that the high mitotic rate and areas of necrosis are reason to worry, but elected to avoid a malignant modifier without definitive evidence such as vascular invasion.
Contributing Institution: Department of Veterinary Medicine, University of Cambridge Cambridge, UK
http://www.vet.cam.ac.uk/

References:
CASE IV: G 8397/12 (JPC 4033557).

Signalment: 18-year-old female putty-nosed monkey, Cercopithecus nictitans.

History: The monkey lived in a German zoo since 2007. After a fight among group members, the animal showed apathy, tachypnea and vomiting. Physical examination under general anesthesia revealed a perforating wound on the right lateral thorax, resulting in severe unilateral pyothorax, which was treated by drainage of the thoracic cavity and repeated wound cleaning, accompanied by administration of antibiotics and analgesics for a couple of days. The monkey showed good response to treatment and initially improved, before its clinical condition deteriorated after 10 days with additional development of neurological signs. Due to poor prognosis, the animal was euthanized and submitted for post mortem examination.

Gross Pathology: Focally extensive within the right ventrolateral chest wall, a chronic, well encapsulated, intramuscular to subpleural abscess was present, reaching from the sixth to ninth intercostal space into the mediastinum with adhesions to the caudal lung lobe and perforation of the costal pleura, accompanied by moderate unilateral fibrinous to hemorrhagic pleural effusion. The right lung showed diffuse necro-suppurative to fibrinous pleuropneumonia with marked compression atelectasis of mainly the caudal parts, whereas the left lung was poorly retracted, hyperemic, and edematous with multifocal miliary to mid-sized abscesses disseminated throughout all lobes. Cerebral as well as cerebellar grey and white matter revealed randomly distributed foci of acute hemorrhagic necrosis, accompanied by diffuse meningeal hyperemia and mild to moderate multifocal to coalescing suppurative meningitis.

Laboratory Results: Aspergillus fumigatus was isolated by fungal culture from the brain.

Histopathologic Description: Throughout grey and white matter as well as within meninges, there are multiple randomly distributed necrotic foci, composed of central debris, sometimes associated with bright eosinophilic material (Splendore Hoepli phenomenon), and surrounded by numerous degenerate neutrophils and macrophages besides fewer lymphocytes and plasma cells. Frequently within necrotic centers, few to large numbers of faintly stained fungal hyphae of approximately 3-6 µm width, characterized by regular septation, thin, parallel walls, and dichotomous, progressive acute angle branching are present. Several small to mid-sized arterial blood vessels within the neuropil contain fibrin thrombi that are often admixed with the fungal hyphae described above, accompanied by moderate to marked fibrinoid change and necrosis of vessel walls. The surrounding tissue shows varying degrees of hemorrhage and lytic necrosis in combination with degenerate neutrophils, foamy macrophages (gitter cells), fewer lymphocytes, and plasma cells as well as moderate adjacent gliosis.

4-1. Telencephalon, putty-nosed monkey: There is a focally extensive area of pallor (malacia) which comprises up to 66% of the section. (HE 7X)

4-2. Telencephalon, putty-nosed monkey: Throughout the area of necrosis, vessels walls are often expanded by numerous neutrophils (vasculitis) and surrounded by edema fluid. Surrounding neuropil is also edematous. (HE 160X)
Contributor’s Morphologic Diagnosis:
Cerebral cortex: Meningoencephalitis, necrotizing, suppurative, acute, multifocal, marked, with multifocal thrombosis, necrosuppurative vasculitis, and numerous intralesional fungal hyphae consistent with Aspergillus fumigatus, putty-nosed monkey (Cercopithecus nictitans), nonhuman primate.

Contributor’s Comment: More than 180 Aspergillus (A.) spp. have been described but only four species (A. fumigatus, A. flavus, A. terreus, A. niger) are commonly associated with invasive infection in primates, with Aspergillus fumigatus being the most common cause (> 90 %) of human pulmonary fungal infections. The uninucleate conidia, or spores, of Aspergillus sp. occur in soil, air, water and greatest numbers are found in hay and straw enriched with leaf and grass compost. They are easily dispersed by the wind and have a diameter small enough (2.5 to 3.5 µm) to reach down to the deep airways. They are considered to be the main vehicle for infective transmission, and when they get the chance to germinate inside the body, producing branched septate hyphae that invade tissues, different forms of aspergillosis can develop. However, the exact portal of entry for the fungal infection could not clearly be identified in the present case. It is possible that Aspergillus conidia entered through the perforating wound and germinated within the thoracic cavity, and from there they invaded the bloodstream and spread to the lung and central nervous system. But it is also possible the infection route was via inhalation of Aspergillus spores, resulting in penetration of distal alveolar spaces. Here, there are optimal environmental conditions for germination into angioinvasive filamentous hyphae that can produce local tissue damage, hemorrhage, infarction, and necrosis.

In healthy, immunocompetent individuals, various elements of the pulmonary innate immune system are involved in recognition and elimination of inhaled Aspergillus conidia, thereby preventing colonization of the respiratory system. Ciliated and mucus secreting epithelial cells perform effective mucociliary clearance that is important for entrapment and elimination of inhaled conidia. Surfactant, mainly produced by type II pneumocytes and Clara cells, has been implicated in antimicrobial activity with surfactant protein A and D serving as collectins. Alveolar macrophages represent first line phagocytic defense by intracellular killing of swollen spores and prevention of germination. Recruited...
neutrophils play an essential role by extracellular (degranulation) as well as intracellular (phagocytosis) elimination of aspergilli. Dectin-1, expressed by macrophages, neutrophils and dendritic cells, is an important receptor of innate antifungal defense being essential for spore recognition and phagocytosis, as well as production of oxygenated free radicals (fungicide al activity). Above that, certain Toll-like receptors (TLR) have been found to play a predominant role in the recognition of *A. fumigatus* (TLR2: recognition of spores, TLR4: recognition of spores and hyphae).8

On the other hand, several pathogenicity factors were found in different *Aspergillus* spp. to overcome certain host defense mechanisms such as endotoxins that inhibit epithelial ciliary activity, as well as a variety of proteases (including elastase, collagenase and trypsin) that damage epithelial cells and, thus, impair effective mucociliary clearance.1,5 Furthermore, *A. fumigatus* produces a phospholipid capable of decreasing the binding of complement factor C3b to its surface, resulting in disturbed complement activation.7 Also other fungal proteins of *A. fumigatus* are probably related to virulence by promoting mycelial growth in lung parenchyma or structural alterations of conidia that are resistant to host defense mechanisms.1

Moreover, it is likely that *Aspergillus* mycotoxins can work as virulence factors due to direct cytotoxic effects. In vitro studies revealed that aflatoxin (produced by *A. fumigatus*) suppresses the function of macrophages, and ochratoxin (produced by *A. ochraceus*) is cytotoxic to lymphocytes and suppresses lymphocytic, monocytic and granulocytic activity. As other possible immunosuppressive mycotoxins, gliotoxin, fumagillin, fumigacin, fumitremorgin A and Asp-hemolysin are discussed while different mycotoxins together may have synergistic effects. However, further in vivo studies are needed for confirmation of direct relation to *Aspergillus* pathogenesis.6 Beyond that, melanin pigment, mannitol, catalases and superoxide dismutases are suggested as antioxidant defenses produced by *Aspergillus*.4 Although it seems that certain antioxidant
molecules produced by *A. fumigatus* do not directly inhibit the oxidizing activity of phagocytes, inhibition of reactive oxygen species production by macrophages (e.g. with high blood cortisol levels or corticosteroid treatment) abolishes their ability to kill the spores while phagocytosis continues so that conidia can germinate and proliferate intracellularly.\(^8\)

However, since pulmonary macrophages and neutrophils constitute a crucial part of first line innate host defense, neutropenia and long-term corticosteroid treatment or hyperglucocorticoidism, as observed in the present case, are generally regarded as major risk factors for the pathogenesis of invasive aspergillosis.\(^1,4\)

**JPC Diagnosis:** Brain, cerebrum: Meningoencephalitis, necrosuppurative, multifocal, severe, with vasculitis, hemorrhage and numerous fungal hyphae.

**Conference Comment:** This is a great case exhibiting the vascular affinity of *Aspergillus* spp. within the brain of this monkey, with its severity alluding to suspicion of an underlying immune compromising condition such as chronic steroid administration. The contributor mentions this may have played a role, and describes the complex interactions of the fungi’s virulence factors with the host’s immune response; an interaction which often allows it to run amok in susceptible patients.

Aspergillosis is perhaps more readily recognized as the cause of granulomatous pneumonia and air sacculitis in avian species, mycotic rhinitis in dogs, abortion in cattle, secondary abomasal ulcers in ruminants following grain overload or mastitis, and hepatocyte megalocytosis and necrosis in dogs.\(^2,3\) The latter is associated with production of aflatoxin of which there are several produced by *Aspergillus* spp. with B\(_1\) being the most significant and best studied example.\(^10\) Toxin production tends to be greatest in stored or unharvested mature grains. Among nonhuman primates, reports of infection are seemingly rare, limited to a single outbreak at the London Zoo in conjunction with tuberculosis. During this outbreak, Old World monkeys were affected by disseminated lesions in the lungs, liver, kidneys and spleen.\(^9\)

**Contributing Institution:** Pathology Unit, German Primate Center, Göttingen, Germany www.dpz.eu

**References:**