The Armed Forces Institute of Pathology
Department of Veterinary Pathology

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CASE I: S 1120/08 (AFIP 3133964).

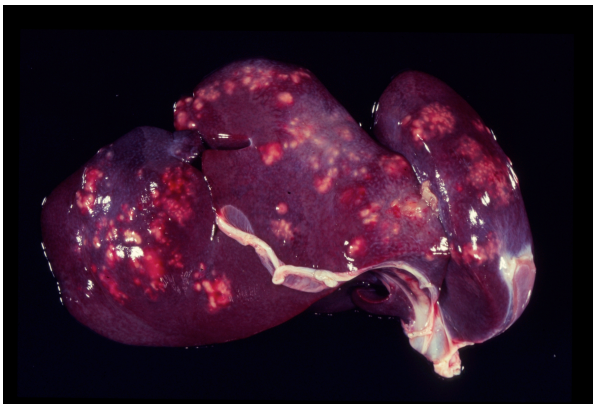
Signalment: 2-year-old, female, Guereza monkey (*Colobus guereza*).

History: The monkey was housed in an open-range recreation park together with numerous other monkeys of the same species and in close contact to other animal species and human visitors. Clinically, a poor body condition, dyspnoea and abdominal discomfort were noticed for two days prior to death.

Gross Pathology: Necropsy revealed a poor body condition and moderate hyperplasia of hepatic and gastric lymph nodes. Approximately 30% of the liver tissue was replaced by multifocal to coalescing, variably demarcated, irregularly shaped, pale yellow, occasionally slightly elevated nodules measuring 0.5 - 4.0 cm in diameter. On the cut surface, they showed a pale yellow coloration and a dry, elastic consistency. In addition, a moderate multifocal fibrino-necrotizing gastritis and mild acute diffuse catarrhal enteritis were observed.

Laboratory Results: Urinalysis revealed a pH value of 8.0; 300 mg protein/liter; a normal amount of glucose and urobilinogen; and no leukocytes, nitrites, ketones or bilirubin.

The liquid of the anterior eye chamber contained a urea concentration of 19.98 mmol/L (120 mg/dL).



1-1. Liver, guereza monkey. Approximately 30% of the liver tissue is replaced by multifocal to coalescing, variably demarcated, irregularly shaped, pale yellow, occasionally slightly elevated nodules of 0.5-4.0 cm in diameter. Photographs courtesy of Department of Pathology, University of Veterinary Medicine, Hannover, Germany, peter.wohlsein@tiho-hannover.de

Aerobic and anaerobic microbiological cultures of the liver resulted in a mild amount of α-hemolytic *Streptococcus* spp. and coagulase-negative *Staphylococcus* spp. Aerobic and anaerobic microbiological cultures of the stomach revealed a mild amount of *Enterococcus* species, coagulase-negative *Staphylococcus* spp., coryneform bacteria, α-hemolytic *Streptococcus* spp., *Geotrichium* species, *Prevotella bivia* and *Prevotella intermedia*. Both organs were culturally negative for *Listeria monocytogenes* under cold enrichment.

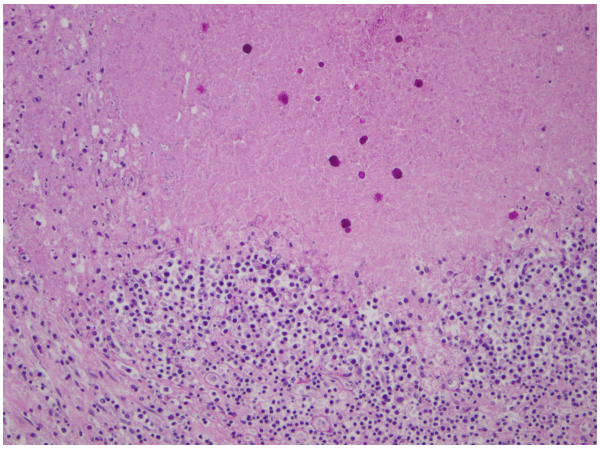
PCR of the liver was negative for human hepatitis virus A, B, C, and *Francisella tularensis*.

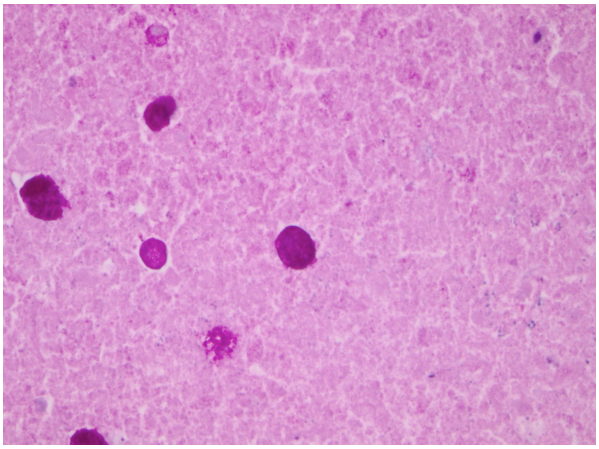
Immunohistology of the liver was positive for *Entamoeba histolytica* and negative for *Toxoplasma gondii*, *Coxiella burnetti* and *Listeria monocytogenes*.

Histopathologic Description: Liver: There are randomly arranged, multifocal to coalescing, irregular to round, pale eosinophilic, hypocellular areas surrounded by a poorly demarcated hypercellular rim. The centrally located pale eosinophilic, floccular mass with an irregularly distributed scant amount of karyorrhectic debris gradually changes into a peripheral zone of dissociated hepatic cells with pale, irregularly vacuolated cytoplasm, loss of cellular detail, ruptured outer membranes, and karyorrhexis and karyolysis. This area is surrounded by a zone composed of an inner layer of macrophages, epithelioid macrophages and few neutrophilic granulocytes, gradually changing into an outer layer consisting of macrophages, lymphocytes, plasma cells and fibroblasts embedded in an extracellular matrix exhibiting a moderate to high amount of collagen fibres that irregularly extends into the neighbouring hepatic tissue. Within the center of the lesions there is a moderate amount of multifocal, oval, indistinct, unicellular structures of approximately 20-30 µm diameter with clear borders, abundant finely vacuolated, lightly eosinophilic cytoplasm, and a single, eccentrically located, small, round, pale amphophilic nucleus (interpreted as protozoan trophozoites). These protozoal organisms are labelled brightly red in periodic-acid-Schiff (PAS)-stained sections. The surrounding hepatic tissue displays a mild to moderately increased amount of periportal fibroblasts and collagen fibre-rich extracellular matrix (periportal fibrosis), a mildly increased amount of periportal bile ducts, and moderately increased amount of intravascular and intrasinusoidal erythrocytes (congestion).

Contributor's Morphologic Diagnosis: Liver: Hepatitis, granulomatous and necrotizing, multifocal to coalescing, chronic, severe with intralesional protozoal trophozoites consistent with *Entamoeba histolytica*.

Contributor's Comment: *Entamoeba (E.) histolytica* is a protozoan parasite belonging to the phylum Sarcomastigophora, subphylum Sarcodina (Rhizopoda), order Amoebida, family Entamoebidae, genus *Entamoeba*. It is the etiologic agent of human amoebiasis, with an incidence of up to 50 million clinical cases per year, including up to 100,000 fatalities.[13,19,21,23] *Entamoeba histolytica* is distributed worldwide among human beings, but is also reported to occur in a wide range of New and Old World monkeys.[5,13,20] It is rarely found in domestic animals, including dogs, cats, cattle and captive macropods.[2,14,15,20] Furthermore, guinea pigs and hamsters are susceptible to experimental infection.[2,3,17] In addition to *E. histolytica*, multiple non-pathogenic species of the genus *Entamoeba*, including *E. dispar* and *E. moshkovskii*, are frequently detected in faeces and the intestinal tract of humans and non-human primates.[13,16,20] The only other pathogenic species of the genus *Entamoeba* is *E. invadens*, which occurs in reptiles.[6] Notably, it is impossible to differentiate the cysts of the highly related species *E. histolytica, E. dispar*, and *E. moshkovskii* by light microscopic investigation of fecal smears.[13,16,19,21,23] However, morphological differences, including a slightly larger size of *E. histolytica* trophozoites (20-30 µm) as compared to *E. dispar* trophozoites (12-15 µm), have been described. Furthermore, only *E. histolytica* is erythrophagocytic and invasive and therefore can be detected within





1-2, 1-3. Liver, guereza monkey. There are multifocal to coalescing, randomly arranged, irregular to round, pale eosinophilic, necrotic areas surrounded by a poorly demarcated, hypercellular rim. Within the center of the lesion are few PAS positive oval, indistinct unicellular structures of approximately 20-30 um in diameter. Photographs courtesy of Department of Pathology, University of Veterinary Medicine, Hannover, Germany, peter.wohlsein@tiho-hannover.de

tissues upon histological examination.[19] In histological sections, *E. histolytica* trophozoites exhibit a granular and lightly stained cytoplasm and a round nucleus with chromatin plaques at the periphery and a small endosome.[6]

Infection with *E. histolytica* occurs via the fecal–oral route. In human patients, excystation takes place in the large intestine. The trophozoites are usually commensals within the intestinal lumen, reproducing by binary fission and encysting as they move further down the digestive tract. Cysts are shed for many months to years in untreated humans. The cysts remain infective for weeks to months in a moist environment.[13,19] A complete intra-intestinal life cycle, including cyst formation, is only reported to occur in humans and non-human primates; therefore, the zoonotic potential of infected non-primate mammals is thought to be limited.[18]

Pathogenicity of *E. histolytica* is affected by the strain of organism, host species infected, nutritional status, environmental factors and bacterial flora of the gastrointestinal tract.[20] Only in cases of mucosal invasion, which happens in less than 10% of human patients, does *E. histolytica* become pathogenic and lead to amoebic dysentery.[13,19] Clinical signs in affected monkeys include apathy, lethargy, weakness, dehydration, gradual weight loss, anorexia, vomiting, and severe diarrhea, which may be catarrhalic or hemorrhagic.[20] Amoebiasis commonly presents as necrotizing colitis in humans and many non-human primates.[5,13,19,20] However, fibrino-necrotizing gastritis seems to be the principal lesion in certain species of leaf-eating monkeys of the subfamily colobinae [old world monkeys (Cercopithecoidea), family cercopithecidae], including colobus monkeys (*Colobus guereza*), silvered leaf monkeys (*Presbytis cristatus*), dusky leaf monkeys (*Presbytis obscurus*), and proboscic monkeys (*Nasalis larvatus*).[4,8-11] The stomach of the colobinae is divided into four parts: presaccular, saccular, tubular, and pyloric portion, with the first two portions serving as enlarged fermentation chambers as a special adaption to the leaf-eating lifestyle. It is suggested that the normal neutral pH within these gastric compartments provides a favourable environment for excystation of ingested *E. histolytica* cysts, followed by tissue invasion.[4,8] Invasive trophozoites are regularly detected within the ulcerative gastric lesions.[4,8-11] In rare cases, and independently of whether the primary lesion is in the stomach or in the colon, some trophozoites are thought to enter the vessels of the mesenteric vasculature, thereby leading to metastatic foci of amoebic infection in distant parts of the body.[20] Fatal amoebiasis with abscess formation, particularly in the liver and more infrequently the lung and the central nervous system, is reported in man, baboons, chimpanzees, orang-utans, spider monkeys, douc langurs, and several colobus monkeys.[4,5,8,11,13,18-20] Amoebic hepatic lesions may become clinically obvious many years after the initial exposure and without concurrent intestinal lesions and fecal cyst shedding.[8,13,18] Similar to the presented case, most reported cases of amoebic hepatic lesions in non-human primates were characterized by a multifocal distribution and granulomatous and necrotizing lesions.[4,8,11,18] The central necrotic areas of the lesions are thought to represent foci of caseous necrosis due to the action of lytic substances produced by the trophozoites.[8] In human patients, however, the liver lesions are mainly situated in the right liver lobe and consist of a single abscess in most patients (65-75%).[13]

The differential diagnosis of granulomatous and necrotizing hepatic lesions in primates includes yersiniosis, salmonellosis, listeriosis, tularemia, tuberculosis, necrobacillosis, Q-fever, histoplasmosis, yellow fever, infections with lymphocytic choriomeningitis virus (callitrichid hepatitis), human hepatitis B virus, herpesvirus simiae (herpesvirus B), herpesvirus saimiri, simian varicella virus, herpesvirus tamarinus, herpes simplex virus, cytomegalovirus, ebola virus, toxoplasmosis, *Capillaria hepatica*, and schistosomiasis. Non-human primates are possible reservoir hosts of human hepatitis A virus; however, they display only slight hepatocellular degeneration, necrosis and inflammation and are most likely clinically asymptomatic. Furthermore, chimpanzees have been experimentally infected with the human hepatitis C virus; however, spontaneous infections of non-human primates have not been reported.[1] Due to the zoonotic potential of most of these diseases, increased personal protection and a thorough etiologic work-up is suggested in cases of hepatitis in non-human primates.

AFIP Diagnosis: Liver: Hepatitis, random, necrotizing and granulomatous, multifocal to coalescing, severe, with amoebic trophozoites.

Conference Comment: Conference participants discussed the virulence factors in *Entamoeba histolytica* infection. The first of three virulence factors is a multifunctional lectin (Gal/GalNAc lectin); in addition to binding to glycoprotein residues of the target cell, it also plays a role in cytolysis, invasion, resistance to complement and possibly the process of encystation. The second virulence factor is the amoebapore, a channel-forming protein analogous to T-cell perforins and NK-lysin from Natural Killer (NK) cells; once the channel is inserted into the host cell membrane, extracellular water and ions rush into the cell causing cell lysis. The final virulence factor is a family of cysteine proteases produced by *E. histolytica*. These proteins function to break down the extracellular matrix, facilitating invasion. Not only does this aid organism invasion, the breakdown of the extracellular

matrix also results in loss of cellular adhesion, possibly contributing to further cellular death.[12]

These virulence factors must enable *E. histolytica* to overcome a variety of innate host defenses. The first and most extensive host barrier is the layer of mucin extending from the oral cavity to the rectum. The glycoproteins found in host mucin competitively bind to residues used in amoebic attachment; resident intestinal bacteria also bind to mucin, further reducing available binding sites to the protozoan. The complement system plays a role in the innate immune response to *E. histolytica*. The neutral cysteine proteinase elaborated by the parasite has been shown to activate complement via the classical and alternative pathways, thus activating the immune response. However, the Gal/GalNAc lectin possessed by the organism binds C8 and C9, thus preventing formation of the membrane attack complex.[12]

In addition to lectin-mediated evasion of the complement system, *E. histolytica* has several methods by which it evades the host immune system. The cysteine proteinases and hydrolytic enzymes not only degrade extracellular matrix proteins, they also degrade IgA and IgG antibodies, thereby facilitating initial invasion of the intestinal epithelium as well as aiding in systemic dissemination.[12] When immune complexes bind to the amoeba, a unique process of capping takes place whereby the bound antibodies are quickly moved to the posterior pole of the organism, the cap is "released" and the plasma membrane is regenerated;[7] thus, immune complexes are essentially shed from the protozoal cell surface. *E. histolytica* is also able to alter the host acute-phase immune response, primarily via an unknown mechanism of T-cell-modulated macrophage function, especially Th1.[12] The amoeba also produce monocyte locomotion-inhibitory factor which inhibits the respiratory burst in macrophages. Research also has demonstrated the presence of surface perioxiredoxin which neutralizes host-generated reactive oxygen species and nitric oxide.[7] Serine proteases secreted by *E. histolytica* bind to cathepsin G secreted by neutrophils, and protozoal arginase consumes host L-arginine, a precursor for the nitric oxide production by host macrophages.[7]

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http://www.tiho-hannover.de/einricht/patho/index.htm

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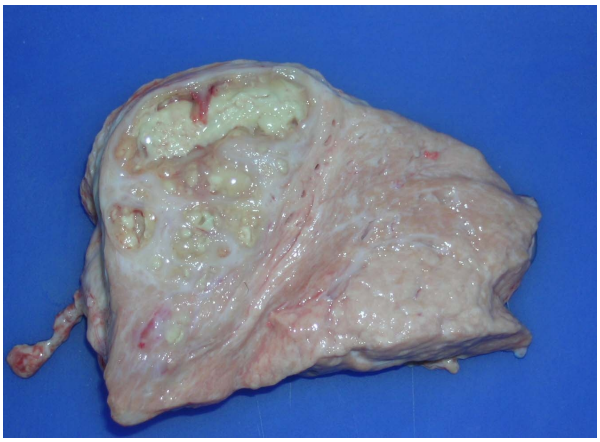
CASE II: 2/10 (AFIP 3165171).

Signalment: 4-year-old, female, Holstein-Friesian, cow (*Bos taurus*).

History: The affected animal belonged to a herd of 122 milking cows with a mean daily milk production of 23 liters. The herd experienced a sudden increase in the incidence of acute clinical mastitis, mainly after parturition. Clinically the outbreak was characterized by drastic decrease in milk production and diminished milk quality with most of the affected cows exhibiting a somatic cell count (SCC) > 1,000,000. Over a period of two months, approximately 20 cows were culled because of the sustained unresponsiveness to antibiotic therapy. No cases of pneumonia or arthritis were noted in association with the episodes of mastitis. Bacteriology performed on milk samples from several of the affected cows yielded the isolation of *Mycoplasma bovis*.

Gross Pathology: Grossly the mammary gland was severely reduced in size and increased in consistency at palpation. The parenchyma was disseminated by multiple small coalescing nodules. The scant material that could be drawn from the most severely affected quarter consisted of very thick yellowish exudate. On cut surface the normal mammary parenchyma was completely obliterated by thick bands of fibrotic tissues. Embedded within the fibrotic reaction were segmentally distended mammary ducts, and recesses of the cysterna were filled and replaced by dense purulent-like yellowish material.

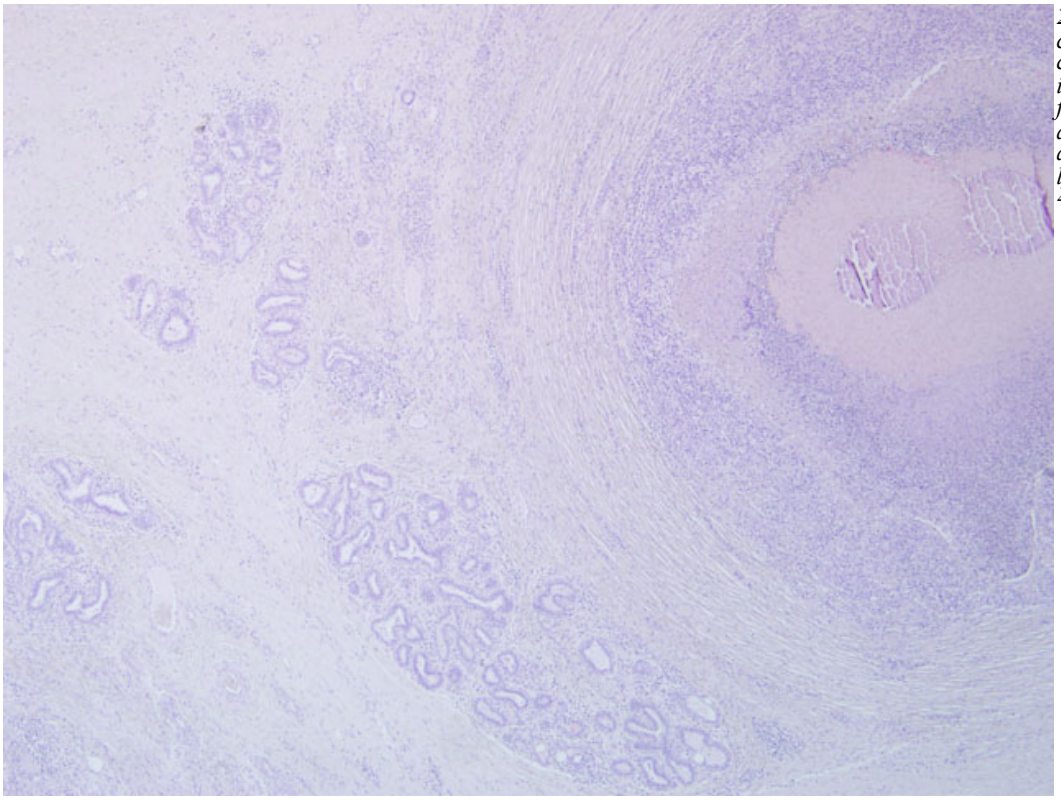
Laboratory Results: Swabs from the affected portions of mammary gland were cultured using routine bacteriological procedures on BHI-agar and MacConkey-agar in a normal atmosphere and on blood agar in a microaerophilic atmosphere. Swabs were cultured also for *Mycoplasma* spp. directly on pleuropneumonia-like organism (PPLO) agar plates. Bacterial culture yielded a massive growth of *Mycoplasma* spp. colonies characterized by the typical "fried-egg" morphology. No other bacterial organisms were identified. The isolates of *Mycoplasma* spp. were confirmed to be *M. bovis* through specific PCR amplification.



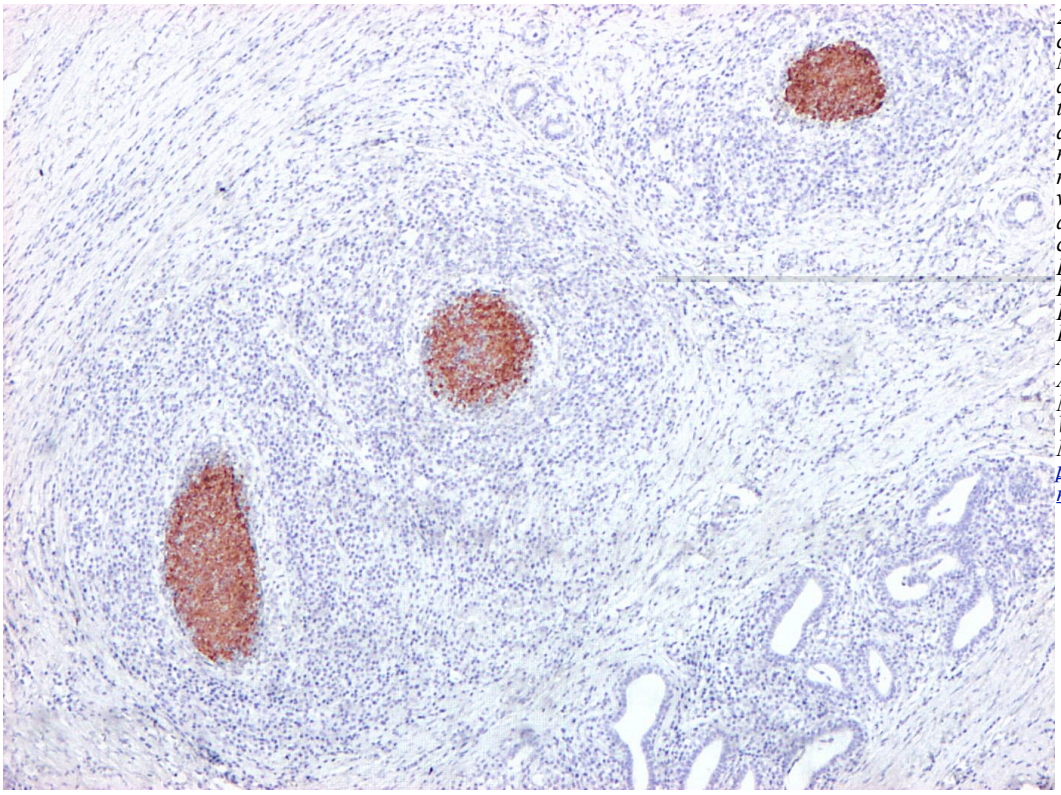
2-1. Mammary gland, cow. The mammary gland contains multiple small coalescing nodules. On cut section, normal mammary gland is obliterated by thick bands of fibrotic tissue. Embedded within the fibrotic reaction are segmentally distended mammary ducts and cysterna that are filled with dense purulent yellow material. Photographs courtesy of Dipartimento di Patologia Animale, Igiene e Sanita' Pubblica, Sezione Anatomia Patologica Aviare, Facolta' di Medicina Veterinaria, Via Celoria 10, 20133 Milano, Italy, paola.roccabianca@unimi.it

Immunohistochemically, abundant *M. bovis* antigen was detected in the cytoplasm of degenerate neutrophils and foamy reactive macrophages or admixed with the necrotic debris. Necrosuppurative foci were surrounded by severe fibrosis with marked infiltration of degenerated neutrophils, CD3-positive T-cells, CD79α-positive B-cells, and CD68-positive histiocytes.

Histopathologic Description: Mammary gland: Multifocal to coalescing inflammatory lesions affect and partially efface 40% of the mammary gland parenchyma. Inflammatory foci are mainly located in the lumen of ducts with complete loss of epithelial lining (necrosis). Inflammatory foci have a multilayered appearance and are characterized by a central area of colliquative necrosis bordered by elevated numbers of karyorrhectic neutrophils, a more peripheral layer of inflammation composed of numerous lymphocytes and plasma cells intermixed with fewer foamy reactive macrophages, and an external fibrous capsule. The less affected lobules are characterized by a moderate number of lymphocytes and plasma cells and fewer eosinophils expanding the interstitium.



2-2. Mammary gland, cow. Multifocal to coalescing areas of inflammation and fibrosis replace glands and ducts, with adjacent large areas of lytic necrosis. (HE 40X)



2-3. Mammary gland, cow. Abundant Mycobacterium bovis antigen is detected in the cytoplasm of degenerate neutrophils, foamy macrophages, or within the necrotic debris. Photographs courtesy of Dipartimento di Patologia Animale, Igiene e Sanita' Pubblica, Sezione Anatomia Patologica Aviare, Facolta' di Medicina Veterinaria, Via Celoria 10, 20133 Milano, Italy, paola.roccabianca@unimi.it

Alveoli are multifocally lined by 2-3 layers of epithelial cells (moderate hyperplasia) and occasionally contain lipid vacuoles. The interlobular septa are expanded by a moderate amount of fibrous connective tissue. In some sections moderate to severe atrophy of alveoli secondary to severe interstitial fibrosis is evident.

Contributor's Morphologic Diagnosis: Mammary gland: Severe, multifocal to coalescing, chronic, necrotizing and pyogranulomatous mastitis with diffuse and moderate fibrosis and atrophy.

Contributor's Comment: *Mycoplasma bovis* infection is associated with a variety of bovine clinical diseases including bronchopneumonia, mastitis, polyarthritis, tenosynovitis, otitis media, myocarditis, meningoencephalitis and reproductive disorders. An increasing number of epidemiological and clinicopathological data underline that *M. bovis* is emerging worldwide as one of the most pathogenic organisms involved in bovine bronchopneumonia. Especially in beef cattle, *M. bovis* infection has been associated with the so-called chronic pneumonia-polyarthritis syndrome (CPPS) and bovine respiratory disease (BRD) complex.[8]

Besides its major role as a respiratory pathogen and as a causative organism of polyarthritis in beef cattle, *M. bovis* is also considered an important agent of mastitis in dairy cows. Mastitis caused by *M. bovis* has been estimated to cost the U.S. dairy industry over USD $100 million annually, with infection rates of up to 70% in some herds, which is even greater than the losses resulting from mycoplasmal pneumonia.[9] Over recent years, with the replacement of classical bacteriological techniques and the development of more reliable and accurate PCR and ELISA-based diagnostic tests for the identification of mycoplasmas, *M. bovis* is being increasingly recognized as a primary agent of mastitis also in Europe.[1] The substantial economic losses caused by *M. bovis* derive from the development of a chronic progressive mastitis with decreased milk production and lower milk quality. Because no efficacious antibiotics or vaccines have been approved for the treatment or prevention of *M. bovis* mastitis, culling is recommended for controlling the disease, even if this drastic measure of control results in considerable animal replacement costs.[10]

Epidemiological and pathogenetic mechanisms responsible for *M. bovis*-induced mastitis are far from being fully elucidated. Ascending and hematogenous routes of infection are both implicated in the development of disease. Fomites, such as contaminated milking equipment or solutions used for intramammary infusion, represent the most documented routes of *M. bovis* transmission among dairy cows. The existence of environmental sources for *M. bovis* and their role in transmission and clinical disease are poorly characterized, although recent investigations pointed out the role of recycled bedding sand as a potential source of *M. bovis*.[6] Secondary colonization of the mammary glands starting from primary foci of bronchopneumonia and polyarthritis or during septicemia has been also postulated as a likely event leading to mastitis. The contrary is also true where the mammary gland acts as a primary focus of infection followed by septicaemia and polyarthritis. Vertical transmission of *M. bovis* with congenital mammary gland infection in prepubertal heifers has been also hypothesized in a recent investigation. In dairy herds, direct galactogenic transmission of *M. bovis* infection from cows with mastitis represents one of the main causes of bronchopneumonia, otitis media and polyarthritis in suckling calves.[4,14]

M. bovis infection of the mammary gland elicits a persistent inflammatory response characterized by the up-regulation of several proinflammatory cytokines and chemokines, complement activation, massive local recruitment of neutrophils and eosinophils and drastic increase in vascular permeability. Despite the sustained inflammation mounted by the host in response to *M. bovis*, several lines of evidence suggest that this reaction is not sufficient to eradicate the pathogen from the mammary gland, and infection usually persists over multiple lactations.[2,7] A similar situation has been also observed in the context of respiratory infections where the ability of *M. bovis* to establish persistent infections characterized by chronic progressive bronchopneumonic lesions may result from an inadequate and ineffective Th-2-polarized immune response.[13]

Although not pathognomonic for *M. bovis* mastitis, the combination of the following clinical features in lactating cows should prompt the suspicion of mycoplasmal infection:

a. drastic rise in bulk and individual milk somatic cell counts;
b. sudden onset of agalactia, with firm swollen and painless quarters;
c. rapid separation of the milk drawn from affected quarters in a floccular precipitate and a watery supernatant;
d. rapid spread of the infection from quarter to quarter;
e. rapid spread of the infection from cow to cow within the affected herds;
f. unresponsiveness to antibiotic therapy;
g. decreased milk production with marked atrophy of affected quarters in clinically recovered cows.

Clinical signs of systemic involvement are generally rare although enlargement of supramammary lymph nodes, fever, anorexia, and concurrent polyarthritis have been reported in several outbreaks.[5,12]

Based on the few and inconsistent data reported in the current literature, the pathology of *M. bovis* mastitis generally consists of an early phase dominated by massive infiltration and/or exudation of granulocytes (both neutrophils and eosinophils) in the edematous lobular interstitium, in the wall of cistern and within the acinoductal luminal compartment. The acute phase is soon followed by chronic progressive changes mainly characterized by proliferation of the affected ductuloalveolar epithelium and gradual interstitial fibrosis accompanied by infiltration of lymphocytes, macrophages and plasma cells. Epithelial erosion/ ulceration in the larger ducts and cisterns may lead to the formation of polypoid proliferations of granulation tissue protruding into and occluding the luminal compartment. The chronic phase progresses to an end stage condition where fibrosis and fibroplasia prevail on the acinoductal epithelial hyperplasia with intense atrophy and scarring of the affected parenchyma.

The few morphological studies reported so far in the literature appear largely inadequate to address the entire spectrum of pathological manifestations associated with *M. bovis* infection of the mammary gland. The unusual case of *M. bovis* mastitis provided best illustrates this concept. In contrast to the pathological features previously described for *M. bovis* mastitis, the lesional picture in this case is characterized by severe chronic necrosuppurative and fibrosing galactophoritis consisting of segmental ectasia of affected mammary ducts with collection of necrotic debris and degenerated neutrophils, formation of multinodular coalescing abscesses/pyogranulomas and intranecrotic foci of dystrophic mineralization. Interestingly, foci of necrosuppurative galactophoritis described share many pathological features with the characteristic *M. bovis*-associated bronchocentric lesions frequently observed in the lungs of beef cattle. These peculiar inflammatory changes possibly reflect a common pathogenesis for lesions originating both from bronchi/bronchioli and mammary ducts.

Gross and microscopic findings similar to those described in our case could be elicited also by other common causes of bovine galactophoritis, including *Mycobacterium bovis*, *Nocardia asteroides*, *Arcanobacterium pyogenes*, *Prototheca zopfii*, and *Cryptococcus neoformans*. However, as confirmed by bacteriological examination, *Mycoplasma bovis* represented the sole bacterial pathogen implicated in our case. Furthermore, specific histochemical stains (Ziehl- Neelsen, PAS and Gram stains) were also applied to rule out other possible agents of bovine galactophoritis. Other less frequent causes of mycoplasmal mastitis in dairy cows include *Mycoplasma californicum, Mycoplasma bovigenitalium* and *Mycoplasma canadense.*

AFIP Diagnosis: Mammary gland: Mastitis, pyogranulomatous, multifocal to coalescing, marked, with fibrosis and glandular atrophy and loss.

Conference Comment: The contributor provides an excellent review of *Mycoplasma bovis*. Based on the chronicity of the lesion, many conference participants favored other etiologies, including *Mycobacterium bovis*; pyogenic bacteria, such as *Arcanobacterium pyogenes*; or higher order bacteria, such as *Nocardia asteroides*. Conference attendees noted that necrotizing lesions typically associated with *Staphylococcus aureus* or *Escherichia coli* were not observed; this stimulated a discussion on the various methods by which to classify pathogens of the mammary gland.

Bacterial pathogens of the mammary gland can be grouped by any one of a variety of criteria. McGavin and Zachary's *Pathologic Basis of Veterinary Disease* suggests dividing the organisms into two groups based on the source of infection to other cows. First are those in which the mammary gland itself serves as the primary source of infection, such as *S. aureus*, *Streptococcus agalactiae*, and *Mycoplasma* species, and cow-to-cow transmission occurs. Second are the coliform organisms, which are acquired from the environment; infection occurs through teat contact with contaminated material or equipment. Finally, *Streptococcus uberis* and *Streptococcus dysgalactiae* form an overlapping group in which the mammary gland and environmental contamination serve as important sources of infection. This type of epidemiologic classification system provides valuable information for the producer and veterinarian regarding disease prevention and treatment.[3]

From a pathologic and pathogenesis perspective, categorizing bacterial mastitis according to the type of lesion produced is helpful in determining an underlying etiology. Gram-negative bacilli produce such lesions as vasculitis, necrosis, hemorrhage and edema, leading to endotoxemia. Gram-positive bacteria typically result in acute necrotizing mastitis or chronic suppurative mastitis. With acute necrotizing mastitis, Gram-positive bacteria, such as *S. aureus*, secrete bacterial products which elicit a massive neutrophilic response that contributes to extensive necrosis which progresses to gangrenous mastitis. In contrast, in chronic suppurative mastitis the pus-forming Gram-positive bacteria, such as *Streptococcus dysgalactiae,* and *Arcanobacterium pyogenes*, invoke a neutrophilic response resulting in suppuration and

fibrosis, which is often centered on lactiferous ducts and sinuses; *Mycoplasma bovis* elicits a similar histologic lesion.[3]

Because many conference attendees considered tuberculous mycobacteria high on the differential diagnosis, a brief outline of the pathologic features of bovine mycobacterial mastitis is provided in the chart below:[11]

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	Portion(s) affected	Gross appearance	Histologic appearance	Spread
Disseminated miliary form	Interacinar areas	1. Caseous nodules 2. Thick fibrous capsule 3. ± mineralization	1. Replacement of lobules by tubercle 2. Interlobular duct lumena expanded by cellular exudate 3. Tubercles in supramammary lymph nodes	Remain localized in affected lobule(s)
Chronic organ form	Entire lobule Intra- and inter-lobular ducts	1. Grey-red to white 2. Bulge on cut surface 3. Smoothly bumpy with a dry appearance	1. Retention of lobular outlines with sparing of interlobular septa 2. Acini obliterated by tuberculous granulation tissue 3. Intra- and inter-lobular duct walls expanded by granulation tissue	Intramammary spread via ducts; no lymph node involvement
Caseous tuberculous form	Entire gland	1. Gland markedly enlarged by irregular caseous areas 2. Hyperemic margin 3. ± areas of chronic organ tuberculosis	1. Fibrin and leukocyte exudation 2. Surrounded by hyperemic granulation tissue 3. ± hemorrhage	Readily spreads to unaffected areas; no lymph node involvement

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CASE III: NEPRC CASE 1 (AFIP 3163068).

Signalment: 5-year-old, male, intact, rhesus macaque (*Macaca mulatta*).

History: This macaque was inoculated with SIVmac239 and had undergone routine phlebotomies. More than a year after inoculation, the animal developed diarrhea, dehydration, and marked weight loss. A weight loss of 2.1 kg (from 8 to 5.9 kg) was recorded over a one month period.

Gross Pathology: The body of this five-year-old, SIV239-infected, male macaque had minimal amounts of body fat. The lymph nodes were enlarged, and the spleen was irregular in shape with mild follicular hyperplasia. No other significant gross lesions were present.

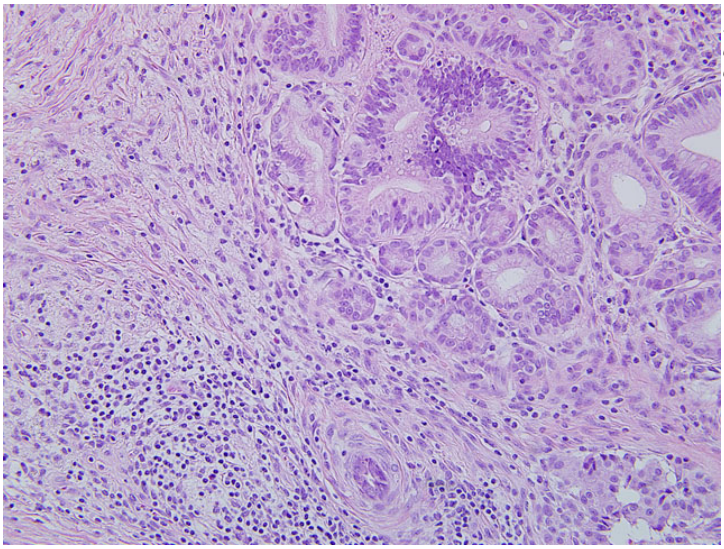
Laboratory Results: Immunohistochemistry (IHC) of liver and pancreas for adenovirus was positive.

Histopathologic Description: Pancreas: Replacing approximately 90% of exocrine pancreatic tissue, sparing only the main pancreatic duct, are multifocal to coalescing areas of necrosis composed of degenerate epithelial cells that are admixed by deposits of fibrin, cellular debris, and fibrosis that often encircle remnant islets of Langerhans. These areas of necrosis are characterized by large numbers of degenerate epithelial cells that have vacuolated cytoplasm and nuclear pyknosis. Scattered acinar cells bordering the regions of necrosis contain prominent, 6-10 µm magenta, round, intranuclear inclusion bodies. Similar inclusion bodies are rarely noted within the ductular epithelium, almost exclusively in small degenerate epithelial cells that are partially exfoliated into the ductular lumen. There is scattered hyperplasia of the remnant exocrine epithelial cells characterized by moderate anisocytosis and scattered mitotic figures. The fibrosis is loosely organized in many areas and is often interspersed with moderate to abundant numbers of lymphocytes and plasma cells with fewer macrophages and degenerate neutrophils. There are scattered hemosiderin-laden macrophages throughout the pancreas. In the peri-pancreatic fat and surrounding blood vessels within the adipose tissue there are small to moderate numbers of lymphocytes and plasma cells.

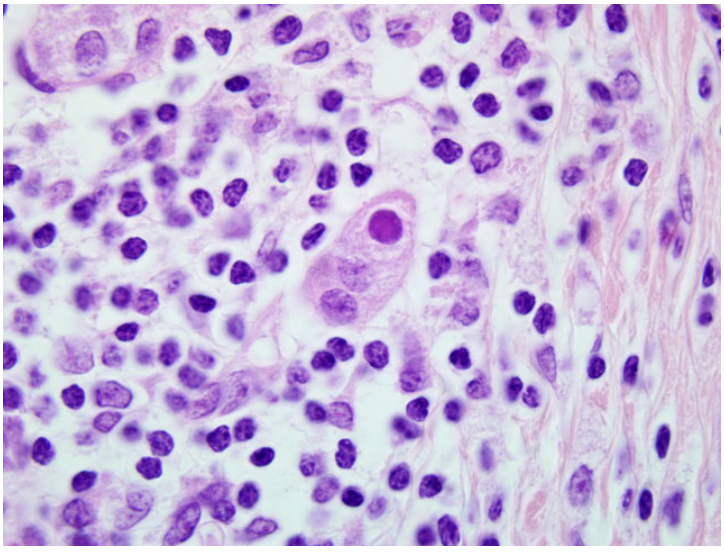
Immunohistochemistry for adenovirus: There are multifocal positive cells noted within the areas of necrosis and in the ductular epithelium. Immunoreactivity is strong and intranuclear. The isotype matched negative control revealed no immunoreactivity.

Contributor's Morphologic Diagnosis: Pancreas: Severe, multifocal to coalescing, chronic, necrotizing and fibrosing pancreatitis with intraepithelial intranuclear adenoviral inclusions.

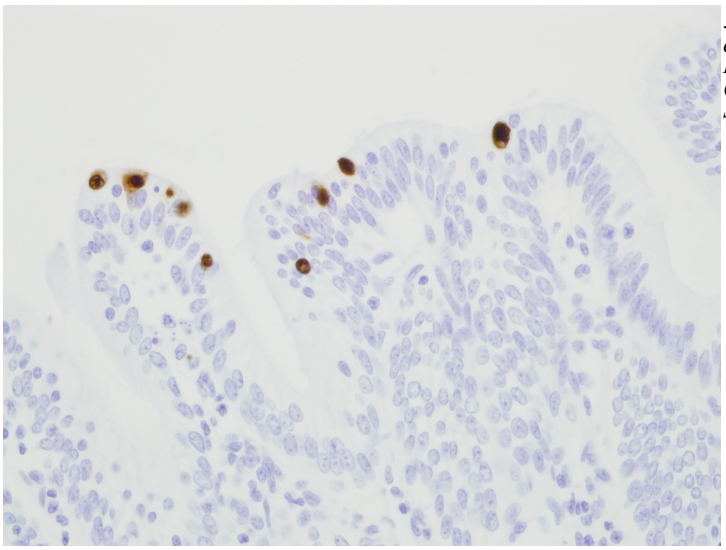
Contributor's Comment: Rhesus adenovirus is a non-enveloped, hexagonal in outline with icosahedral symmetry, 80-100 nm in diameter, double-stranded DNA virus belonging to the Adenoviridae family and Mastadenovirus genus.[8,11] A sizeable number of simian adenoviruses (SAdVs) that infect Old World monkeys and chimpanzees have been characterized. Approximately 24 simian adenovirus prototypes are recognized and additional isolates are currently awaiting better characterization. The majority of simian adenoviruses have been isolated from *Macaca* spp., *Cercopithecus aethiops*, *Papio cynocephalus*, and *Saimiri sciureus*.[7] The viral genome consists of a single linear molecule of double-stranded DNA, 36 to 44 kbp in size. Viruses replicate in the nucleus and their replication is facilitated by extensive modulation of the host immune response. Virions are composed of 252 capsomers: 240 hexons that occupy the faces and edges of the 20 equilateral triangular facets of the



3-1. Pancreas, rhesus macaque. Surrounding pancreatic ducts are loosely arranged fibrosis admixed with moderate to abundant lymphocytes and plasma cells. (HE 200X)



3-2. Pancreas, rhesus macaque. Within epithelial cells there are rare eosinophilic intranuclear inclusion bodies. (HE 1000X)



3-3. Pancreas, rhesus macaque. Pancreatic ductular epithelium show multifocal strong nuclear immunopositivity. Photograph courtesy of New England Primate Research Center, Harvard Medical School, One Pine Hill Drive, Southborough, MA 01772, Andrew_Miller@hms.harvard.edu

icosahedrons and 12 pentons that occupy the vertices. From each penton projects a penton fiber 20 to 50 nm in length, with a terminal knob.[11] Roughly 40 proteins are coded for by the viral genome and are transcribed following a complex RNA splicing. The structural proteins make up the hexons, penton, penton fibers, and other associated virion structures.

Adenoviruses are capable of establishing chronic infections following an initial exposure that may or may not be clinically relevant.[13] All of the adenoviruses have narrow host ranges. Many cause acute respiratory or gastrointestinal disease but also may cause persistent infection with periods of latency that become reactivated upon immunosuppresion.[6] In dogs and other canids, canine adenovirus-1 causes infectious canine hepatitis that produces acute necrosis and inflammation in the liver often accompanied by grossly visible edema of the gallbladder.[4] Canine adenovirus-2 is one of the known causative agents of canine infectious tracheobronchitis, which is commonly referred as " kennel cough".[9] Canine adenovirus type 2 can cause pneumonia in dogs which is clinically mild unless complicated with secondary bacterial infections.[9] Acute rhinitis that is manifested as part of general respiratory disease can be caused by both adenovirus type 1 and 2.[9] Adenoviruses are pneumotropic and enterotropic in ruminants.[6,9] Equine adenovirus type 1 (EAdV1) causes upper respiratory tract disease, follicular conjunctivitis, bronchopneumonia, and infection of the gastrointestinal tract. EAdV1 is peculiarly associated as a dominant pathogen in the uniformly fatal, inherited disease syndrome known as primary severe combined immunodeficiency disease (PSCID). The foals are born devoid of B and T lymphocytes and a consistent and dominant feature of PSCID is an inexorably progressive EAdV1 bronchopneumonia.[14] Mice are host to two distinct adenoviruses: mouse adenovirus type 1 (MAV-1), causing hemorrhagic foci of necrosis in multiple organs and wasting disease (especially in nude and SCID mice); and mouse adenovirus type 2 (MAV-2), which is asymptomatic and does not exhibit clinical disease.[12]

In rhesus macaques, adenovirus infection is one of the most common opportunistic infections to accompany terminal cases of SIV infection. Adenoviral pancreatitis can also be seen in animals that are immunosuppressed for organ transplantation and in neonates. Adenovirus associated disease in rhesus macaques is most commonly found in the small and large intestine, the liver and gallbladder, and the pancreas. The pancreatic lesion is often fulminant with abundant necrosis making organ identification difficult. If the pancreatitis persists for long periods of time the marked epithelial hyperplasia and proliferation that occurs can be confused with a pre-neoplastic change.

Chronic pancreatitis is characterized by destruction of exocrine tissue and is typically accompanied by fibrosis, parenchymal atrophy, and in late stages with destruction of endocrine parenchyma.[3,4] Chronic pancreatitis may present as repeated bouts of acute pancreatitis, the major distinction being an irreversible impairment of pancreatic function with the former. Chronic inflammation of the pancreas with mostly lymphoplasmacytic infiltrations is seen most commonly in the dog, but does occur in cat, horse and cattle.[4]

The most frequently reported pancreatic disease in nonhuman primates is diabetes mellitus secondary to the deposition of islet amyloid polypeptide (amylin) in the Islets of Langerhans. In addition, pancreatitis and islet destruction in nonhuman primates can be induced by certain drug treatments, such as streptozotocin, and interference with the pancreatic duct and blood supply.[10] Prior to the discovery of SIV as a major immunocompromising pathogen in rhesus macaques, spontaneous pancreatitis was reported rarely in nonhuman primates, and at least two cases were reported in rhesus monkeys with adenoviral inclusions.[1,2,5,10] The spontaneous nature of these early reports of adenoviral pancreatitis is dubious, as it is highly likely that an as yet unrecognized pathogen likely caused immunosuppresion predisposing to adenoviral disease in these animals. In cases of pancreatitis in nonhuman primates, especially rhesus macaques, it is imperative to determine if adenovirus played a role and if the animal was immunocompromised.

AFIP Diagnosis: Pancreas: Pancreatitis, necrotizing, chronic, diffuse, severe, with fibrosis, exocrine acinar atrophy and loss, ductular hyperplasia, and rare epithelial intranuclear inclusion bodies.

Conference Comment: Some slide variation exists, with some sections containing areas of lytic necrosis and others characterized by extensive fibrosis with little discernible pancreatic tissue.

Participants discussed the relationship between the location of viral inclusion bodies within the cell and the properties of the viral genome. In general, DNA viruses tend to replicate in the nucleus and produce intranuclear inclusion bodies, *e.g.* herpesviruses, adenoviruses, parvoviruses, etc. One notable exception to this generality is the Poxviridae family, which induce eosinophilic intracytoplasmic inclusion bodies. In the dog, few RNA viruses produce viral inclusion bodies visible by light microscopy, the most notable of which include: rabies virus, which results in the classic intracytoplasmic Negri body within infected

neurons; and canine distemper virus, a morbillivirus that can result in both intranuclear and intracytoplasmic viral inclusion bodies.

The contributor provides an excellent overview of the viral properties of the *Adenoviridae,* comparative pathology of adenoviral infection, and pancreatic disease in nonhuman primates.

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http://www.hms.harvard.edu/nerprc/

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CASE IV: N09-1 (AFIP 3134310).

Signalment: 12-year-old, male, castrated, mixed-breed horse (*Equus caballus*).

History: The horse was housed on pasture with five other horses. Its diet consisted of orchard and brome grass hay and 12% sweet feed. The water source was a flowing river. Vaccinations for Eastern equine encephalitis (EEE), Western equine encephalitis (WEE), and tetanus were current.

The owners noted depression and dysuria. Following three days of progressive signs to include lethargy and decreased thirst, the referring veterinarian examined the animal. A fever of 103.3°F was recorded, along with a decreased appetite, trouble prehending hay and grain, and teeth grinding. The veterinarian treated the animal with sulfa antibiotics and flunixin meglumine (Banamine™). The next day the horse was more lethargic, reluctant to move, and had a temperature of 101.5°F.

On day 4, the horse was referred to the University of Tennessee (UT) Large Animal Clinic. Upon presentation, the horse was depressed and lethargic, had a weak gait, and dragged both toes of the pelvic limbs at the walk. The neurologic exam revealed a grade III-IV weakness of all four limbs with variable ataxia. The tongue tone was weak, especially to the right, and the horse had difficulty prehending food. There were intermittent fasciculations of the facial muscles, but no other cranial nerve deficits were noted. Additionally, the mucous membranes were icteric and injected, and the horse had multiple abrasions on the tongue and lips and hemorrhages on the gums. Rectal examination, abdominocentesis, equine infectious anemia titers, and upper airway endoscopy were within normal limits. Gastroscopy revealed small ulcers along the lesser curvature of the stomach. Treatments at the UT College of Veterinary Medicine included DMSO, flunixin meglumine (Banamine™), trimethoprim sulfa, IV fluids, dexamethasone, penicillin, and gentamicin (Gentocin™). Despite therapies, the horse became more ataxic and weak, to the point of falling down. He was maintained in the sling overnight, and by the morning of the 6th day, he was head pressing and completely unaware of his surroundings.

Gross Pathology: The horse was humanely euthanized and presented for necropsy to the UT Department of Pathobiology. Thirty-six hours later, the brain was removed and half was submitted for rabies IFA testing and half was placed in formalin. The trigeminal ganglion appeared swollen. The pituitary was enlarged and had several raised tan masses (adenomatous hyperplasia) on the capsular surface.

Laboratory Results: Clotting factors and biochemistry were within normal limits. A complete blood count revealed an HCT of 27%. A lumbosacral cerebrospinal fluid (CSF) tap on day 5 failed to detect abnormalities.

Histopathologic Description: Pituitary gland, pars nervosa: There was marked lymphocytic perivascular cuffing with infiltration into the adjacent neuropil affecting the pars nervosa. The tissue was markedly expanded by edema, and the perivascular cuffing also involved the vasculature of the pars intermedia, which is generally of even thickness, although there is multifocal cystic degeneration with accumulation of a brightly eosinophilic acellular material.

Contributor's Morphologic Diagnosis: Pituitary gland, pars nervosa: Severe subacute lymphocytic neurohypophysitis (encephalitis) with neuronal necrosis and spongiosis.

Contributor's Comment: The histologic lesions in this case are consistent with the diagnosis of viral encephalitis. Negri bodies, the pathognomonic lesion of rabies virus infection, were not observed in this case. The absence of Negri bodies can occur in cases where the animal is euthanized before the disease has run its full course. Immunohistochemistry, with appropriate controls, was performed on sections of the pituitary gland and thalamus/ hippocampus by the Cornell Veterinary Diagnostic Laboratory, and viral antigen/Negri bodies were not detected. The IFA assay performed on fresh brain (half submitted) was positive. IFA is the gold standard in antemortem rabies diagnoses; when used on fresh brain tissues, it consistently detected 100% of rabies-positive archival cases.[7]

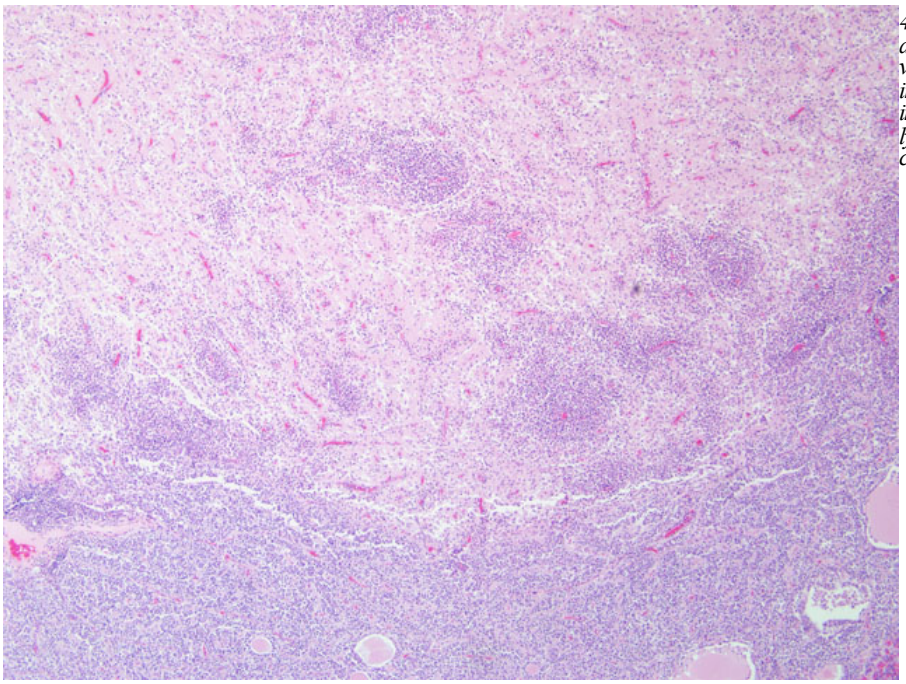
Rabies is a zoonosis with one of the highest fatality rates.[3] The rabies virus belongs to the genus *Lyssavirus* of the Rhabdoviridae family and is classically spread by a bite from an infected animal.[10] The virus replicates locally before moving along peripheral nerves, where it binds to the nicotinic acetylcholine receptors at the neuromuscular junctions. The virus moves via peripheral nerves toward the central nervous system (CNS) by retrograde axoplasmic transport.[10] Once in the spinal cord, movement occurs using both anterograde and retrograde axoplasmic flow. The virus can also move along peripheral nerves so that the salivary glands become involved, allowing transmission to occur through saliva.[10] This early spread of virus allows for dissemination of infection often before severe clinical signs and immune responses develop.

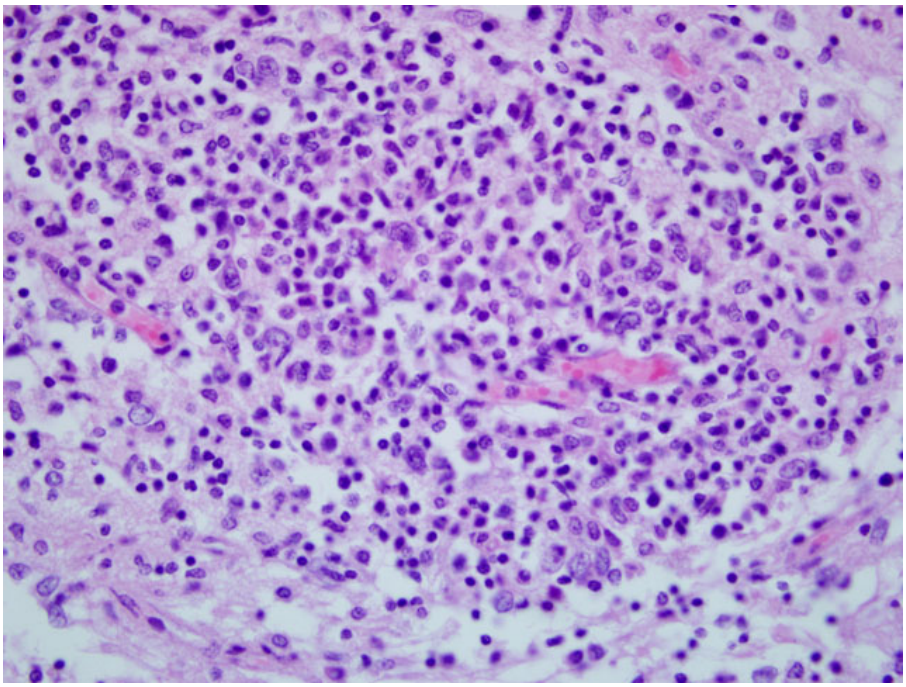
There are typically no gross lesions in rabies cases. Microscopic lesions are variable and include non-suppurative leptomeningitis with perivascular cuffs, neuronal degeneration and neuronophagia, gliosis, malacia of the spinal cord grey matter, and ganglioneuritis. Infected neurons may contain intracytoplasmic acidophilic inclusions (Negri bodies), which, while pathognomonic for rabies, are not present in all cases. Negri bodies tend to occur in large neurons, particularly in the pyramidal neurons of the hippocampus, neurons of the medulla oblongata and Purkinje cells of the cerebellum. Hence, these locations are preferred for microscopic or fluorescent antibody diagnosis of rabies.

Negri bodies were identified in the brain of 10/21 horses examined in one large study.[3] It was thought that they occurred more commonly in horses that survived longer than 4 days.[3] Negri body size may be affected by length of clinical disease and stage of infection when the animal was euthanized.[2] Detection of Negri bodies with hematoxylin and eosin and Sellers stain is limited, as it detected only 50-80% of positive samples, gave false positive results, and had reduced effectiveness in autolyzed samples.[1]

Dr. Adelchi Negri identified the intracytoplasmic inclusions in infected neurons that bear his name in 1903. Even today, the true significance of these structures is still mysterious. It seemed to Negri that fixed strains (passaged in the laboratory) of rabies were less likely to produce Negri bodies because neurons were destroyed early and that the street strains (natural disease) favored their formation, partly because they caused less significant neuronal damage.[6]

Rabies in horses in particular has been associated with a spectrum of clinical signs that include, but are not limited to, ataxia, recumbency, pharyngeal paralysis, fever, hyperesthesia, loss of tail and/or anal sphincter tone, progressive paresis, muscle tremors, sweating, anorexia, colic, and lameness.[3] Paresis and hind limb ataxia are the most commonly observed clinical signs.[3] In equine cases, where a CSF tap is performed, pleocytosis with lymphocyte prominence and fewer mononuclear cells, macrophages, and neutrophils is documented. The clinical signs may be related to the concentration of inoculated virus, the pathogenicity of the strain, and the proximity of CNS tissue to the site of inoculation. The spinal cord form and dumb form





4-1, 4-2. Pituitary gland, pars nervosa and pars intermedia, horse. Surrounding vessels in the pars nervosa and extending into the neuropil and adjacent pars intermedia are moderate numbers of lymphocytes, macrophages, and plasma cells. (HE 40X, HE 400X)

are much more common than the furious form in horses.[3]

Differentials for CNS disease in horses include vertebral malformation, trauma, infections, abiotrophy, and degenerative or idiopathic lesions. Viruses to consider include rabies, EEE, WEE, St. Louis encephalitis, Louisiana virus, snowshoe hare virus, Cache Valley virus, and Main Drain virus.[5] In one large study, wobbler syndrome and equine protozoal myelitis were the most common diagnoses.[5] The nicotinic acetylcholine receptors have been proposed as the rabies virus receptors.[3] The virus may influence secretion of neuro-modulators and thereby induce functional impairments at sites remote from the site of viral replication. Toll-like receptor 3 has also been implicated in the spatial arrangement of rabies virus-induced Negri bodies and overall success of viral replication.[9] Viruses can exploit cellular proteins for their own benefit.[9]

Immunoperoxidase has been determined to be a useful, accurate, and rapid method for rabies diagnosis.[1] Immunoperoxidase is thought to be more sensitive in early diagnoses of suspected cases in which conventional histology and IFA might not detect viral antigens. In a small case series of four IFA-confirmed rabies cases, IMHC on paraffin tissue detected all rabies cases. Only 2/4 cases had Negri bodies.[5] Similarly, immunoperoxidase on 40 rabies cases showed a specificity of 100% and a sensitivity of 97.6%. Additionally, in another case series, 39/40 cases were positive for rabies with immunoperoxidase, making it a reliable diagnostic technique.[4] Negri bodies were seen in only a fraction of these cases (10/17).[4] Viral antigens were detected in dendrites, axons, glial cells, granular cells in Ammon's horn, pyramidal cells and pericaryons of neurons in stratum gangliosum and stratum granulosum of cerebellar cortex.[1]

The amount of rabies antigen varies depending on location within the brain. Following testing of 252 confirmed rabies cases, the thalamus, pons, and medulla were the most reliable parts of brain for testing. Previously, the hippocampus was recommended due to the need to find large inclusion bodies, which occurred at the highest frequency at this site.[2] Current recommendations suggest that the hippocampus and brainstem be sampled.[2]

AFIP Diagnosis: Pituitary gland, pars nervosa and pars intermedia: Hypophysitis, perivascular, lymphohistiocytic and plasmacytic, multifocal, moderate, with gliosis.

Conference Comment: The contributor provides an excellent discussion of rabies infection, pathogenesis, clinical signs and diagnostics.

Several participants included rabies virus infection in their differential diagnosis list along with other causes of viral encephalitis in the horse. There are two biotypes of rabies virus: fixed virus and street virus. As mentioned by the contributor, the fixed virus is stable and used for developing vaccine strains. The street virus is the "wild-type" virus involved in disease outbreaks.

Upon inoculation of a susceptible animal, the virus initially replicates in myocytes before budding from the muscle cell and infecting nerves at the neuromuscular junction.[10] The rabies virus glycoprotein receptors for neuronal cell adhesion molecule (NCAM) and the p75 neurotrophin receptor convey the neurotropism displayed by the virus.[8] Retrograde axonal transport may involve virus phosphoprotein interaction with microtubule motor protein dynein LC8. Once the virus reaches the CNS, transmission of the virus between neurons results in ascending and descending spread of the virus and precipitating the typical clinical signs and pathologic changes.[10]

The exact mechanism by which rabies causes neuronal lesions and ultimately death is unknown. One possibility is marked viral-induced down-regulation of genes in the brain; affected genes are often involved in regulating cellular metabolism, growth and differentiation. Elevation in the nitric oxide content in rabies-infected brains suggests nitric oxide neurotoxicity. Mouse models of rabies infection demonstrate virus-induced apoptosis, another possible mechanism of neuronal injury and lesions.[10]

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