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Department of Veterinary Pathology
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Conference Moderator:
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CASE I: H-8401 (AFIP 3107575).

Signalment: 24-year-old female chimpanzee (*Pan troglodytes*).

History: Six days prior to death the chimpanzee was stiff, hesitant to move its neck and held its head down. The differential diagnosis included trauma or a neurologic disease. A complete blood count and clinical chemistry panel on the day following presentation revealed a mild leukocytosis and moderate hypokalemia. Radiographs of the cervical region were noncontributory, although physical examination of the area elicited a pain reaction even under anesthesia. Antibiotic and anti-inflammatory therapies were instituted with waxing and waning of results over the next six days and a relatively rapid decline culminating in death.

Gross Pathology: Numerous light brown foci (5-11 mm) were present on the surface (dorsal and ventral) and extending into the neuropil of the brain. Since this animal was housed in an outdoor corral, cross sections of the brainstem and a portion of the hippocampus were submitted for rabies diagnosis (negative).

Laboratory Results:
Day 1: Leukocytosis (16.3 k/µl); neutrophilia (13.3 k/µl); and hypokalemia (2.6 mEq/L).
Day 3: Leukocytosis (35.5 k/µl); neutrophilia (31.2 k/µl); and hypokalemia (2.5 mEq/L).
Day 4: Leukocytosis (28.7 k/µl); neutrophilia (22.4 k/µl); and hypokalemia (2.8 mEq/L).
Day 5: Leukocytosis (28.0 k/µl); neutrophilia (22.2 k/µl); and hypokalemia (2.7 mEq/L).

Histopathologic Description: The brain contains a large area of hemorrhage and necrosis with admixtures of neutrophils, mononuclear cells and multinucleated giant cells; cellular debris; fibrinoid necrosis of blood vessels; astrocytosis; and protozoal organisms consistent with *Balamuthia mandrillaris.* Trophozoites measure 30-60 µm. Also observed is a diffuse mild infiltration of mononuclear cells in the meninges and perivascular spaces.

Contributor's Morphologic Diagnosis: Brain: Multifocal, marked, necrohemorrhagic and pyogranulomatous meningoencephalitis with fibrinoid vasculitis and protozoal organisms consistent with *Balamuthia mandrillaris.*

Contributor's Comment: Balamuthiasis is an emerging disease of humans and animals with fatal consequences.(4) Originally isolated and identified from a fatal case of meningoencephalitis in a pregnant mandrill (*Papio sphinx*), *Balamuthia mandrillaris* is a free-living amoeba; however, it was not recovered from the environment until 2003.(1,2,5) Encephalitis caused by free-living amoeba (e.g. *Acanthamoeba* and *Balamuthia*) is primarily a problem of immunocompromised patients, although immunocompetent patients are affected by both *Balamuthia* and *Naegleria*.(6) Granulomatous amoebic encephalitis associated with *Acanthomeba* and *Balamuthia* is typically slow in developing and insidious, and a hematogenous route of entry has been hypothesized but not proven for these disease agents.(6) By contrast, the rapidly fatal primary amoebic encephalitis associated with *Naegleria* is associated with exposure to freshwater lakes and swimming or water skiing with entry through the olfactory neuroepithelium.(6) The fact that this animal had a ruptured eardrum leads to somewhat of a quandary as to how the agent entered. Lesions in the brain appeared to represent a hematogenous spread; however, no parasitic lesions were observed outside of the central nervous system, and the presence of an open eardrum would appear to provide an open portal of entry.

AFIP Diagnosis: Brain: Meningoencephalitis, necrohemorrhagic, histiocytic and neutrophilic, focally extensive, severe, with vasculitis, fibrin thrombi, and many amoebae.

Conference Comment: In a diagnostic setting, it is often important, but sometimes difficult, to determine whether vascular inflammatory lesions represent true vasculitis or are simply "innocent bystander" vessels caught within foci of inflammation. The conference moderator emphasized the importance of evaluating vessels distant from the most severely affected areas when making this histologic assessment. In this case, conference participants noted striking endothelial hypertrophy, inflammatory cells transmigrating and occasionally disrupting vessel walls, and perivascular accumulations of gitter cells both in severely and less affected areas of the section, and therefore agreed with the contributor's diagnosis of vasculitis.

This case was reviewed in consultation with Dr. Christopher Gardiner, Consulting Parasitologist for the AFIP's Department of Veterinary Pathology; he noted that the large size of the *Balamuthia mandrillaris* trophozoites is helpful in distinguishing it from those of *Naegleria fowleri* and *Acanthomoeba* sp., the two other free-living amoebae most commonly implicated in human and animal disease. Only one case of amebic encephalitis has been attributed to a fourth free-living amoeba, *Sappinia diploidea*. During the conference, participants reviewed the free-living amoebae, emphasizing the important distinguishing morphologic features. *Acanthamoeba* sp. and *B. mandrillaris* are closely related to one another phylogenetically, but are distant from *N. fowleri* and *S. diploidea*. While most *B. mandrillaris* trophozoites are uninucleate, binucleate forms may be seen, and the presence of multiple nucleoli may be useful in differentiating *B. mandrillaris* from *Acanthamoeba* sp. Although trophozoites are more numerous than cysts, the presence of cysts in brain tissue is useful in excluding *N. fowleri*, as only *B. mandrillaris* and *Acanthamoeba* sp. form cysts in the brain. However, the absence of cysts does not exclude a diagnosis of BAE, as cyst formation is not consistent, as the present case illustrates. When present, the cyst wall of *B. mandrillaris* is unique in that it is three-layered (i.e. composed of an outer ectocyst, inner endocyst, and intervening mesocyst) and lacks pores, whereas the cyst walls of *Acanthamoeba* sp. and *N. fowleri* are two-layered with pores.(3,4)

As mentioned by the contributor, differences in epidemiology and pathophysiology are also useful in differentiating the diseases caused by free-living amoebae. *Acanthamoeba* sp. generally only produces encephalitis in immunocompromised patients, whereas *Balamuthia* amebic encephalitis (BAE) is reported in both the immunocompetent and immunocompromised. By contrast, *N. fowleri* causes primary amebic meningoencephalitis (PAM) in immunocompetent children and young adults, classically within days of exposure to warm fresh waters. Primary amebic meningoencephalitis due to *N. fowleri* has also been reported naturally in bovids and a tapir (see WSC 2007-2008, Conference 22, case IV) and experimentally in a number of mammalian species. Of note, most humans with BAE present with characteristic skin lesions that predate central nervous system signs; such lesions are not characteristic of PAM produced by *N. fowleri*. Intriguingly, individuals of Hispanic origin are overrepresented among human BAE cases in the United States; unique environmental exposures and genetic predispositions have been proposed, but an explanation for this disparity has not been definitively proven. Among animals, BAE is most common in nonhuman primates, but has also been diagnosed in dogs, a sheep, and a horse.(3,4)

The major features that characterize and distinguish the pathogenic free-living amoebae are summarized in the following table, adapted from Schuster and Visvesvara:(3)

Pathogenic Free-living Amoebae			
Feature	***Balamuthia mandrillaris***	***Acanthamoeba* sp.**	***Naegleria fowleri***
Diseases	Balamuthia amebic encephalitis (BAE); cutaneous and sinus infections	Amebic encephalitis; cutaneous and sinus infections*	Primary amebic meningoencephalitis (PAM)
Risk factors	Immunocompromised status (also occurs in immuno-competent); breaks in skin contaminated with soil	Immunocompromised status	Activity in warm fresh waters; diving; not associated with immuno-compromised status
Incubation period	Weeks to years	Weeks to months	Days
Trophozoite stage	12-60 um	15-30 um	15-30 um
Flagellate stage	Not found	Not found	Flagellate stage with 2 flagella
Cyst stage	3-layered wall lacking pores; 10-30 um diameter; cysts form in brain tissue	2-layered wall with pores; 10-15 um diameter; cysts form in brain tissue	2-layered wall with pores; 7-15 um diameter; cysts do not form in brain tissue
**Acanthamoeba* sp. also is associated with amebic keratitis in immunocompetent humans; risk factors include soft contact lens wear and wearing contact lenses while swimming; incubation period is days (versus weeks to months in systemic infections).			

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CASE II: A098567 (AFIP 3136043).

Signalment: 5-month-old, female, mixed-breed dog (*Canis familiaris*).

History: This puppy, purchased in Texas, but living in Indiana at presentation, was evaluated for chronic respiratory distress (present since purchase at 8 weeks of age) and a 2-month history of progressive neurologic dysfunction. Littermates had responded to antibiotic therapy for *Bordetella* infection, but this puppy's respiratory condition had not improved. Serum titers were positive for canine distemper virus. Radiographically, the lungs had a diffuse interstitial pattern. A cerebral mass was detected in the frontal/parietal lobes by magnetic resonance imaging. Histologic evaluation of a biopsy specimen of the cerebral mass resulted in a diagnosis of granulomatous amebic encephalitis. The dog died about 36 hours after the cerebral biopsy procedure and was presented for necropsy examination.

Gross Pathology: Gross lesions were found in the brain and thoracic cavity. A friable, dark-brown to tan, 1.3 cm in diameter, roughly spherical mass extended from the dorsal aspect of the right frontal lobe of the cerebrum into the thalamus. The thymus was atrophied. Both lungs were over-expanded, firm, and mottled tan to red-brown. Indistinct, pale gray to tan, coalescing nodules, 1 to 3 mm in diameter, were evident in cross-section.

Laboratory Results: Frozen lung specimens were submitted to Dr. Visvesvara at the Centers for Disease Control and Prevention (CDC) for identification of the amoebae. The lung was positive by immunofluorescence and real-time PCR for *Acanthamoeba* spp., and negative by both tests for *Balamuthia mandrillaris* and for *Naegleria fowleri*.

Pseudomonas aeruginosa and *Escherichia coli* were isolated from the lung by bacterial culture. The lung and spleen were positive by immunofluorescence for canine distemper virus, and negative for canine adenovirus, herpesvirus, and parvovirus. No virus was isolated from the lung, spleen or brain.

Histopathologic Description: The submitted sections of lung are representative of the pulmonary lesions. Individual and coalescing, poorly demarcated nodules (up to 2 mm in diameter) of granulomatous inflammation were scattered through the pulmonary parenchyma. The centers of the nodules had undergone amorphous to fibrinoid necrosis with scanty hemorrhage. Alveolar spaces in necrotic (central) and viable peripheral zones of the nodules were partially filled with neutrophils, macrophages, fibrin, and numerous amoebic trophozoites with fewer cysts. Trophozoites were spherical to ovoid, ranged from 7 to 19 μm in maximal dimension (mean, 14 μm), and had a large nucleus with a prominent karyosome and abundant vacuolated cytoplasm. Encysted amoebae had amphophilic cyst walls, 1-2 μm in thickness, with space between the exocyst and endocyst.

Where granulomatous inflammation extended to the visceral pleura, overlying mesothelial cells were hypertrophied. Between nodules of granulomatous inflammation, the lung was congested and edematous with increased numbers of alveolar macrophages. Eosinophilic inclusions were observed in the cytoplasm (and less commonly in the nucleus) of bronchiolar epithelial cells and alveolar macrophages; these were labeled immunohistochemically with antibody to canine distemper virus. In other sections (not a feature in the submitted section), sloughed bronchiolar epithelial cells had large amphophilic intranuclear inclusions that were labeled immunohistochemically with antibody to canine adenovirus.

Contributor's Morphologic Diagnosis: 1. Multifocal granulomatous pneumonia with intralesional amebic trophozoites and cysts.
2. Histiocytic alveolitis with eosinophilic cytoplasmic and intranuclear inclusions.

Contributor's Comment: Canine distemper viral infection may have resulted in immunosuppression that predisposed this puppy to other infections. Acanthamoebiasis was considered the most important of these and the cause for granulomatous encephalitis and pneumonia. Amoebae were not detected in tissues other than brain and lung. However, the puppy also had histologic evidence of pulmonary infection with canine adenovirus and oral candidiasis.

Acanthamoeba, *Balamuthia*, and *Naegleria* species are the free-living amoebae that have been associated with encephalitis and disseminated infection in dogs and humans.(2) In histologic sections, recognition of nuclear features, such as the prominent karyosome and lack of peripheral chromatin, is useful in distinguishing amebic trophozoites from macrophages. In this case, the presence of cysts in addition to trophozoites in infected tissues tended to eliminate *Naegleria* from the differential diagnosis, but definitive diagnosis of *Acanthamoeba* infection was based on immunofluorescence and PCR results (performed on lung specimens). Some reported canine cases of acanthamoebiasis have had granulomatous encephalitis and pneumonia (1), and it has been proposed that pulmonary infection is the result of inhalation or aspiration of the organism from water with hematogenous extension to the brain. However, amebic encephalitis has also been recognized in a dog with widely disseminated acanthamoebiasis in which no organisms were detected in the lungs.(2)

AFIP Diagnosis: 1. Lung: Pneumonia, pyogranulomatous, multifocal, severe, with necrosis and many amebic trophozoites and few amebic cysts.
2. Lung: Pneumonia, bronchointerstitial, diffuse, moderate, with alveolar histiocytosis, type II pneumocyte hyperplasia, viral syncytia, and few bronchiolar and histiocytic intranuclear and intracytoplasmic viral inclusion bodies.

Conference Comment: Like several others evaluated in recent WSC sessions, this case demonstrates the importance of searching for an underlying cause of immunosuppression upon the identification of an opportunistic pathogen. In this case, the extensive pyogranulomatous nodules in the lung were clearly evident to conference participants as the predominant lesion. Following description of the most striking lesions, the conference moderator encouraged participants to carefully examine the remainder of the lung; closer examination, beyond cursory subgross perusal, reveals diffuse bronchointerstitial pneumonia attributed to canine distemper virus infection. Slide variation is present, and the characteristic intranuclear and intracytoplasmic eosinophilic viral inclusions are rare in some sections, underscoring the utility of molecular diagnostics (e.g. immunohistochemistry) as employed in this case. Additional microscopic findings noted by conference participants include multifocal hypertrophy of the pleural mesothelium, and in some sections, small aggregates of extracellular and intrahistiocytic coccobacilli (consistent with the bacterial culture results reported by the contributor).

Conference attendees compared and contrasted cases I and II of this conference, and continued the discussion of pathogenic free-living amoebae, as summarized in the conference comment for case I. This case differs from case I by the presence of numerous cyst forms, the walls of which are Periodic Acid-Schiff (PAS)-positive. Additionally, systemic acanthamoebiasis is associated almost exclusively with immunosuppression, as noted above, whereas balamuthiasis occurs in both immunocompromised and immunocompetent hosts. Primary amebic encephalitis caused by *Naegleria fowleri* is not associated with immunocompromise. Interestingly, *Acanthamoeba* keratitis is a disease of immunocompetent humans associated with corneal trauma or improper contact lens hygiene, and carries a far better prognosis than does *Acanthamoeba* encephalitis.(3)

We thank Dr. Christopher Gardiner, Consulting Parasitologist for the AFIP's Department of Veterinary Pathology, for reviewing this case.

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CASE III: T09-1217 (AFIP 3149412).

Signalment: 8-year-old, captive-bred, male rhesus macaque (*Macaca mulatta*).

History: This rhesus macaque was on a pharmacology protocol with indwelling vascular catheters and ports. The catheter and port had to be moved several times due to loss of catheter patency and infection. The animal was placed on long term antibiotics and found down in its cage. Bacterial culture of the port area was positive for coagulase-positive *Staphylococcus* spp. resistant to all fluoroquinolones. The animal was febrile with a holosystolic heart murmur. Clinical pathology revealed mild anemia, leukocytosis and neutrophilia with a left shift (see below). The animal was given fluids and cefazolin but it died overnight.

Gross Pathology: Externally, there was a 1 cm diameter ulcerated cutaneous lesion on the right thigh adjacent to the tunneled area for the vascular line and the point of entry into the femoral vessel. The lungs were diffusely congested and edematous with blood tinged froth in the trachea and nares. The stomach contained red tinged mucus. Multifocal areas of the liver were firm and paler than adjacent tissue. The kidneys were bilaterally enlarged with multifocal yellow-white foci throughout. There were "chicken fat" clots in both ventricles of the heart. The right atrioventricular valve had an adherent vegetative lesion and a fibrotic valve leaflet. The vena cava contained a large thrombus. The spleen was firm and meaty. There was moderate acute hemorrhage within the meninges.

Laboratory Results: CBC and clinical chemistry: PCV 30%, WBC 16 k/µl (PMN 90% with 5% bands), BUN 28 mg/dL, creatinine 0.9 mg/dL, ALT 131 U/L, total protein 6.7 g/dL; Microbiology of port area: *Staphylococcus* spp., coagulase positive, enrofloxacin resistant.

Histopathologic Description: Kidney: Diffusely, the renal cortex demonstrates numerous prominent, large glomeruli and a focal area of infarction with inflammatory effacement of the parenchyma. The infarcted area is composed of several enlarged and obliterated glomerular capsules filled with primarily neutrophils that efface the glomerular tufts and extend into the surrounding tubules. Bacterial (coccoid) colonies can sometimes be seen in these abscessed glomeruli. There is multifocal hemorrhage and coagulative necrosis of adjacent tubules characterized by bright eosinophilic cytoplasmic staining, loss of nuclei and retention of tubular shape. Diffusely the glomeruli are enlarged, with increased numbers of glomerular tufts; capillaries and occasional glomeruli also demonstrate increased eosinophilic matrix. Some glomeruli demonstrate increased periglomerular fibrosis and synechiae. Multifocally there are aggregates of lymphocytes in the interstitium and random increases of interstitial fibrous connective tissue. An arcuate vessel is occluded by eosinophilic fibrillar material and surrounded by subacute inflammation.

Contributor's Morphologic Diagnosis: Kidney, bilateral:
1. Infarction and abscessation, multifocal, subacute, severe with coagulative tubular necrosis and bacterial (cocci) colonies.
2. Glomerulopathy, membranoproliferative and mesangioproliferative, diffuse, severe with mild sclerosis.
3. Nephritis, interstitial, lymphoplasmacytic, chronic diffuse, mild.
4. Thrombosis, arcuate artery, focal.

Contributor's Comment: The acute pulmonary edema was likely the cause of death in this animal, interpreted to be caused by zealous fluid administration with impaired kidney clearance, endocarditis and pulmonary thrombi. The cardiovascular lesions were the direct result of chronic indwelling catheterization with subsequent infection. There was disseminated thrombosis involving multiple organs including the liver, heart, cerebrum, and mesentery. The renal lesions were likely the result of both vascular disease and chronic inflammation.

Indwelling catheters are convenient tools for the administration of compounds tested for pharmaceutical safety and effectiveness research. Cynomolgus monkeys, rhesus monkeys and baboons are commonly used these studies, often involving psychoactive compounds with subsequent behavioral testing paradigms. Catheters may be tolerated successfully for years if appropriate care is taken to prevent infection. The use of vascular access ports has reduced the numbers of infections due to the closure and healing of the skin, preventing casual exposure. However, ports or extravasated materials into the port pocket may cause focal necrosis with ulceration and exposure of the apparatus.

The formation of infarcts associated with thrombosis and embolization is not as difficult a concept as the pathogenesis of immune complex glomerulonephritis. Several texts have covered this subject extensively, and participants are encouraged to review the mechanisms of membranous, membranoproliferative, and mesangioproliferative glomerular changes.

Several studies of indwelling catheters in baboons have revealed infarcts, septic embolic nephritis and mesangioproliferative glomerulonephritis. Bacteria isolated were *Aciteobacter* (*Herella*) sp., *Streptococcus* sp., *Kleibsiella* sp., *Staphylococcus* sp., and *Providencia* sp. Immunofluorescence studies in six animals on frozen sections revealed granular deposits of IgG (6/6), IgM (5/6), C3 (4/6), and IgA and C4 (2/6) in glomerular lesions. Additionally the IgG deposits correlated with the severity of the lesions. Bacterial antigens were seen in three of six cases, strongly suggesting immunological mediation.(3) Baboons in a second study demonstrated renal and hepatic impairment related to long-term catheterization. The renal lesion in that study was described as membranoproliferative glomerulonephritis with dense deposits noted ultrastructurally in a variety of locations, with mesangial cell interpositioning and foot process fusion. These alterations were found in conjunction with the isolation of *Staphylococcus aureus* from the blood and catheters.(4) Sheep have been used to study renal infarcts and chronic indwelling catheters. Bacterial species isolated differed from those found in primates and humans, but membranoproliferative glomerulonephritis and mesangial immune complex deposition were present in clinically asymptomatic sheep.(6)

In a retrospective study of renal tissue from 62 humans with confirmed infective endocarditis, common renal lesions noted were localized infarcts in 31%, and acute glomerulonephritis in 26%. The most common type of glomerulonephritis was vasculitic, without deposition of immunoproteins in glomeruli. Of the renal infarcts, over

half were due to septic emboli, mostly in patients infected with *Staphylococcus aureus*. Acute interstitial nephritis was found in 10% but was more common in biopsy material and seemed attributable to antibiotics. Renal cortical necrosis was found in 10%.(5) In a recent study of long term venous access devices (n=102), it was determined that cultures taken from the inflamed pocket surrounding the implanted port were as reliable as cultures from the catheter tip on removal. The major organisms cultured were coagulase negative *Staphylococcus* sp., *Candida*, and *Staphylococcus aureus*.(2)

AFIP Diagnosis: 1. Kidney: Nephritis, cortical, suppurative, acute, multifocal, moderate to severe, with focally extensive coagulative necrosis (infarction), fibrin thrombi and few Gram positive cocci.
2. Kidney: Glomerulonephritis, membranoproliferative, global, multifocal, moderate, with synechiae, glomerular senescence, chronic interstitial nephritis and fibrosis.
3. Ureter: Ureteritis, chronic, diffuse, mild.

Conference Comment: This case, which illustrates an important complication associated with the use of vascular ports, provided conference participants with the opportunity to describe a challenging slide featuring concomitant chronic and acute processes. The conference moderator encouraged participants, who were not provided with the full signalment and history, to develop and propose a logical pathogenesis to explain the lesions. Primary learning points included: 1) recognizing that the acute cortical nephritis was of hematogenous origin, which in a diagnostic setting should prompt a search for the primary nidus of infection (e.g. in the heart valves or a vascular port) and disseminated lesions in other capillary beds (e.g. in the lung, spleen, and liver); and 2) identifying membranoproliferative glomerulonephritis (MPGN) and properly interpreting it as a chronic lesion, which should also prompt an investigation into the underlying cause. The moderator and conference participants interpreted the tubular lesions, described by the contributor, as most likely secondary to the glomerular lesions.

Numerous specific human glomerular diseases have been characterized in exquisite detail, and an exhaustive review is beyond the scope of these proceedings. In general, glomerular diseases are classified into three broad categories: primary glomerulopathies, systemic diseases with glomerular involvement (i.e. secondary glomerulopathies, e.g. systemic lupus erythematosis [SLE], diabetes mellitus, amyloidosis, bacterial endocarditis, vasculitis), and hereditary disorders (e.g. Alport syndrome, thin basement membrane disease, Fabry disease). Primary glomerulopathies include, among many others, postinfectious glomerulonephritis, rapidly-progressive (crescentic) glomerulonephritis, membranous glomerulopathy, and minimal-change disease.(1) A superb example of crescentic glomerulonephritis was recently examined in WSC 2009-2010, Conference 3, case IV.

Immune mechanisms, including both cell-mediated and antibody-associated, are thought to underlie most primary and many secondary glomerulopathies. The two best-characterized antibody-associated mechanisms of glomerular injury are: 1) antibodies reacting to antigens within the glomerulus; and 2) deposition of circulating antigen-antibody complexes in the glomerulus. In the former, antigens may be intrinsic to the glomerular basement membrane (GBM) proper, or "planted" in the glomerulus from the circulation. For example, in Goodpasture syndrome, the noncollagenous domain (NC1) of the α_3 chain of type IV collagen, intrinsic to the GBM, is the antigen responsible for classic anti-GBM antibody-induced glomerulonephritis. By contrast, myriad antigens, including bacterial products, aggregated immunoglobulins, and nuclear proteins, may be "planted" in the GBM; antibodies then bind to these antigens, resulting in the formation of immune complexes *in situ*. Circulating immune complex glomerulonephritis differs from anti-GBM glomerulonephritis in that the immune complexes lack immunological specificity for the glomerulus; rather, they form outside of and localize in the glomerulus due to a variety of physiochemical and hemodynamic factors. The culpable antigens that initiate immune complex formation may be exogenous (e.g. products of infectious agents) or endogenous (e.g. SLE).(1)

Morphologically, glomerulopathies are often evaluated using immunofluorescence microscopy and electron microscopy. Two patterns of immune complex deposition are noted using immunofluorescence: 1) granular, which is characteristic of both circulating immune complex deposition and immune complexes formed in situ against "planted" antigens; and 2) linear, which is characteristic of classic anti-GBM glomerulonephritis. An understanding of the structures comprising the glomerular capillary wall is essential to properly interpreting the ultrastructure of immune complex glomerulonephritis. The glomerular capillary lumen is bounded by fenestrated endothelial cells, which is further subtended by the GBM, which is composed primarily of type IV collagen, laminin, heparin sulfate, and several other glycoproteins. The GBM has a thick, electron-dense, central lamina densa, and inner and outer, thinner, electron-lucent peripheral layers, the lamina rara interna and lamina rara externa, respectively. The GBM is surrounded by visceral epithelial cells, also known as podocytes, which have foot processes (pedicels) that abut the basement membrane and are separated by 20-30 nm wide filtration slits, each bridged by a thin diaphragm. Finally,

mesangial cells, which are contractile, phagocytic cells of mesenchymal origin, are interposed between the glomerular capillaries and support the glomerular tuft. Ultrastructurally, immune complexes are electron-dense deposits which can be localized specifically to one or more of the following sites: subendothelial (i.e. between the fenestrated glomerular capillary endothelium and the GBM), intramembranous (i.e. within the basement membrane), subepithelial (i.e. between the pedicel and GBM), epimembranous (i.e. upon the GBM interposed between adjacent pedicels), or mesangial (i.e. within the mesangial matrix). Localization of immune complex deposition ultrastructually is useful diagnostically, because many glomerulopathies are characterized by immune complex deposition specifically in one or more of these locations.(1)

Membranoproliferative glomerulonephritis, also known as mesangiocapillary glomerulonephritis, may be secondary to other systemic disorders (i.e. secondary MPGN) or idiopathic (i.e. primary MPGN), and is further divided into types I and II based on ultrastructural, immunofluorescent, and pathologic findings. In humans, the majority of MPGN cases are type I, characterized by discrete subendothelial electron-dense deposits ultrastructurally, and C3 deposition in a granular pattern by immunofluorescence. The pathogenesis of type I MPGN involves activation of both the classical and alternative complement pathways; IgG, C1q, and C4 are also often present. In the less common type II MPGN, also known as dense deposit disease, the lamina densa of the GBM contains an irregular, ribbon-like, extremely electron-dense material. Immunofluorescence demonstrates C3 in an irregular granular or linear pattern on either side of the lamina densa within the GBM, but not within the dense deposits. The pathogenesis is thought to involve activation of the alternative pathway; IgG, C1q, and C4 are usually absent.(1)

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CASE IV: S 34/05 (AFIP 3135084).

Signalment: 4-day-old, male, crossbred, domestic pig (*Sus scrofa domestica*).

History: This piglet was part of a study and was experimentally infected with a recombinant porcine coronavirus (rTGEV). At 24 hours post-infection, the animal was bright and alert and was humanely destroyed.

Gross Pathology: At necropsy, there was diffuse mesenteric edema in the colon. No further gross lesions were observed and reported.

Laboratory Results: CBC: WBC 11.1 k/µl (↑), neutrophils 8.87 k/µl (80.2 %), lymphocytes 1.48 k/µl (13.4 %), monocytes 0.623 k/µl (5.63 %), eosinophils 0.002 k/µl (0.014 %), basophils 0.086 k/µl (0.779 %), RBC 4.56 M/µl, hemoglobin 10.2 g/dl(↓), HCT 30.7% (↓), MCV 67.3 fl (↓), MCH 22.4 pg (↓), MCHC 33.3 g/dl, RDW 26.9 % (↑), platelets 884.0 k/µl (↑↑), MPV 12.2 fl; mild leukocytosis; mild anemia.

Bacteriology: Lungs: *S. aureus* +, *S. intermedius* +; kidney: *E. coli* ++; liver: *E. coli* +; spleen: negative; duodenum: *Bacteroides* spp. +; jejunum: *Bacteroides* spp. +, *Streptococcus* spp. (+); ileum: *Streptococcus* spp. (+); colon: *E. coli* +, *Streptococcus* spp. +, *Bacteroides* spp. ++.

Fluorescent antibody test (FAT): Direct immunofluorescence on cryosections of the small intestine was negative for coronavirus, but positive for rotavirus antigens.

Virology: By RT-PCR a 433 bp long fragment of genome segment 6 of group A rotavirus was amplified in RNA obtained from a jejunum sample.(6)

Electron Micrograph Description: Small intestine, mid-jejunum, fixed in glutaraldehyde, transmission electron micrograph, 11,800X: The image is composed of at least six intestinal absorptive epithelial cells which are characterized by numerous, even, long, plush microvilli along their luminal borders. The terminal web, especially of the two most lateral absorptive cells, shows immediately beneath the microvilli, few electron-dense filamentous structures extending into the cores of the microvilli. Within the cytoplasm there are numerous mitochondria, abundant rough endoplasmic reticulum (RER), occasional lysosomes and few spherical electron-dense vesicles. Three nuclei are present. The lateral cellular borders of the slightly interdigitating enterocytes show apical junctional complexes. Between two absorptive cells there is one goblet cell liberally endowed with characteristic electron-lucent mucous granules. In the center of the image there are two absorptive cells with more electron-lucent and brightened cytoplasm and a disorganized terminal web. Most strikingly is the severe dilation of the RER which has almost completely lost the ribosomes (ribosomal detachment). Within the dilated cisternae of RER there are numerous electron dense viral particles. The microvilli of these cells appear to be less dense and slightly out of register; mitochondria show lysis of cristae and a moderately condensed matrix.

Inset: Within the dilated cisternae of the RER numerous virus particles with envelope and mature virions after loss of the envelope, with a diameter of 60-80 nm, are present with ultrastructural features identical with those of rotaviruses (47,000X).

Contributor's Morphologic Diagnosis: Jejunum: Absorptive cell, cytoplasmic brightening, dilation of RER with abundant rotavirus particles.

Contributor's Comment: Rotaviruses belong to the family *Reoviridae* and are clustered in the genus *Rotavirus*. Infectious rotaviruses are large, nonenveloped, nearly spherical particles with up to three concentric capsid layers. The shells surround a central protein core which contains the viral genome. Most rotaviral preparations contain both intact, complete particles resembling wheels (Latin: *rota*), and particles which have lost the outer capsid layer. The diameter of intact particles is 65-68 nm; particles with only the inner capsid layer have a diameter of approximately 60 nm. The diameter of the core is approximately 38 nm.(2)

Rotaviruses are the causative agents of severe gastroenteritis in animals and humans and also have the potential to be transmitted between species. They are one of the most important infectious agents of severe diarrhea of infants and are associated with approximately 870,000 deaths per year in children under three years of age in developing countries.(2) Rotaviruses infect differentiated epithelial cells of the villi in the jejunum of a multitude of species including calves, piglets, foals, lambs, and avian species. The results are destruction of enterocytes at the apices of the villi of the small intestine, atrophy of the infected villi, restricted absorption, and increased secretion after neurogenic stimulation with a massive loss of liquid and electrolytes. The main clinical signs are diarrhea ("white scours"), weakness and anorexia. Young animals may die as a result of dehydration or secondary bacterial infection. (7)

Infections with rotaviruses are economically important, especially in younger animals one to eight weeks of age. In calves and pigs, lethal infections are seen during the first months of age. In contrast, infections of human newborns are usually relatively mild and asymptomatic. In humans, an increase in the incidence of infections and the severity of the clinical signs with fever and anorexia is seen not before several months of age. Most of the rotavirus infections with gastroenteritis in children are seen between the ages of six months and two years.(2)

Rotavirus is a nonenveloped virus; therefore, its stability and infectivity at temperatures of 18-20 °C is very high. Rotaviruses are also relatively resistant to treatment with formaldehyde, ether or detergents. Viral particles may remain infectious in the environment for several years.(2)

The genome of rotaviruses consists of double stranded, segmented RNA. The entire genome has 11 segments and in general a molecular weight of 11-12 x 10^6. Differences in the migration pattern of the single genome segments after elecrophoresis can be used for classification. Four groups (I-IV) of these electropherotypes can be differentiated. A

distinct feature of rotavirus morphogenesis is that subviral particles, which assemble in the cytoplasmic viroplasms, bud through the membrane of the RER, and maturing particles are transiently enveloped. This is one of the most interesting aspects of rotavirus replication.(2,3)

Serologically, rotaviruses are divided into seven distinct groups (A-G). There is no correlation between the electropherotype and the serotype or group specificity of rotaviruses. Within the genus *Rotavirus* most of the characterized rotavirus isolates harbor a common group antigen. They are classified as group A. Isolates with similar replication characteristics, but which lack the group specific antigen are classified in additional groups (e.g. B, C, D, and E). Due to numerous additional characteristics of isolates belonging to one of the five groups, rotaviruses are subdivided into several subtypes. Rotavirus infections in cattle most often belong to group A. In humans infections are caused by viruses of group A or B. Rotavirus infections in pigs are often caused by viruses belonging to group B, C, or E. Infections in birds belong mainly to group D. Due to the large variety of rotavirus serotypes and subtypes present in the different species, animals and humans can be infected within a short time period with different serotypes.(4)

AFIP Diagnosis: Small intestine, absorptive epithelial cells: Degeneration, with dilated endoplasmic reticulum and intracytoplasmic round virions.

Conference Comment: The contributor provides a useful appraisal of the entity, and readers may find it helpful to compare the pathogenesis of rotaviral infection with that of parvoviral infection, reviewed in WSC 2009-2010, Conference 16, case II. The diarrhea that results from rotaviral infection is attributed to three mechanisms: 1) malabsorption due to enterocyte necrosis and villus atrophy; 2) villus ischemia and activation of the enteric nervous system by a vasoactive agent released from infected enterocytes; and 3) rotaviral production of nonstructural protein 4 (NSP4), which acts as a secretory enterotoxin.(1) Therefore, both malabsorption and hypersecretion of fluid and electrolytes likely contribute to diarrhea and dehydration seen in rotaviral enteritis.

In addition to "three week scours" or "white scours" in pigs, there are several other distinct syndromes in animals attributed to rotaviral infection, including infectious diarrhea of infant rats (IDIR), enzootic diarrhea of infant mice (EDIM), and diarrhea in young rabbits, calves, lambs, foals, puppies, kittens and poultry. Among these syndromes, rotaviral infection is generally characterized by several consistent themes, including a propensity to cause clinical disease in only young animals, a tendency to cause mild disease, and a predilection for infection of enterocytes at the villar tips.(1,5) That said, several intricacies regarding rotaviral infection in certain species warrant elaboration. In rats and mice respectively, IDIR and EDIM are both characterized by diarrhea in neonates less than two weeks old, with only subclinical infection in older animals. Infectious diarrhea of infant rats is caused by an atypical (i.e. non-Group A) rotavirus that is likely of human origin. In addition to intestinal villus attenuation and enterocyte necrosis that is typical of other rotaviral infections, IDIR is characterized by epithelial syncytia that are considered pathognomonic and may contain eosinophilic intracytoplasmic viral inclusions. In both wild and laboratory mice, EDIM is caused by a single, highly contagious strain of Group A rotavirus and results in transient diarrhea that may or may not be clinically significant.(5) In both lambs and rabbits, rotaviral infection is often seen in combination with bacterial co-pathogens (e.g. *E. coli*); lambs are unique in that viral infection of the colon may occur in this species, whereas in other species the small intestine is affected, with the specific site (i.e. duodenum, jejunum, or ileum) varying by species.(1,5)

Most conference participants considered coronaviral enteritis (i.e. transmissible gastroenteritis [TGE]) in the differential diagnosis for this case. The signalment and clinical signs of coronaviral and rotaviral infections are analogous, although the latter is generally less severe and less frequently characterized by vomiting. The gross and histopathologic findings are also comparable between the two entities, and both cause lesions whose severity is inversely proportional to age in piglets. Similarly, the microscopic lesions in the small intestine of calves with rotaviral enteritis are identical to those of calves with coronaviral enteritis; however, the former does not cause colonic lesions. Coronaviruses have a single-stranded RNA genome, are enveloped, and measure slightly larger than rotaviruses, ranging from 70-200 nm in diameter and averaging 100-130 nm in diameter. The characteristic "corona" of peplomers for which coronaviruses are named is best visualized ultrastructurally in negatively stained preparations.(1)

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