Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE
2020-2021

Conference 10

2 December, 2020

Joint Pathology Center
Silver Spring, Maryland

CASE 1: 17-13-2 JT75 (4140283-00)

Signalment:
Adult, male Rhesus macaque (*Macaca mulatta)*

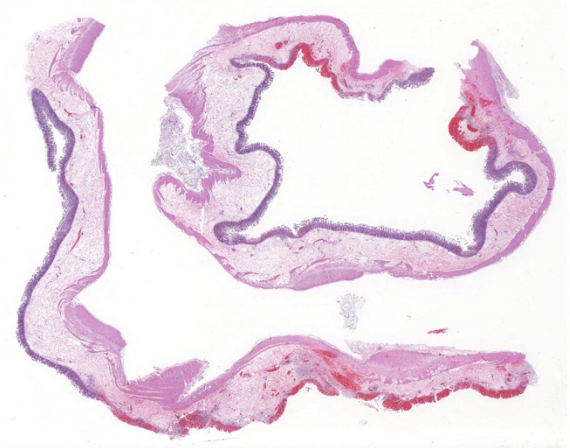
History:
This Rhesus macaque was part of a study to evaluate different regimens of supportive care after intramuscular exposure with Ebola virus. Intensive supportive care provided to this animal included intravenous fluids, antibiotics and corticosteroids. Despite supportive care, this animal died fourteen days after viral exposure.

Gross Pathology:
At necropsy, there was a focally extensive area of mucosal hemorrhage of the descending colon, approximately 10 cm from the anus. There were multifocal, focally extensive areas of mucosal hemorrhage in the cecum, gastric fundus and proximal duodenum. The liver was diffusely swollen and friable. Clear yellow fluid was present in the pericardial sac, thoracic cavity, abdominal cavity and the scrotum.

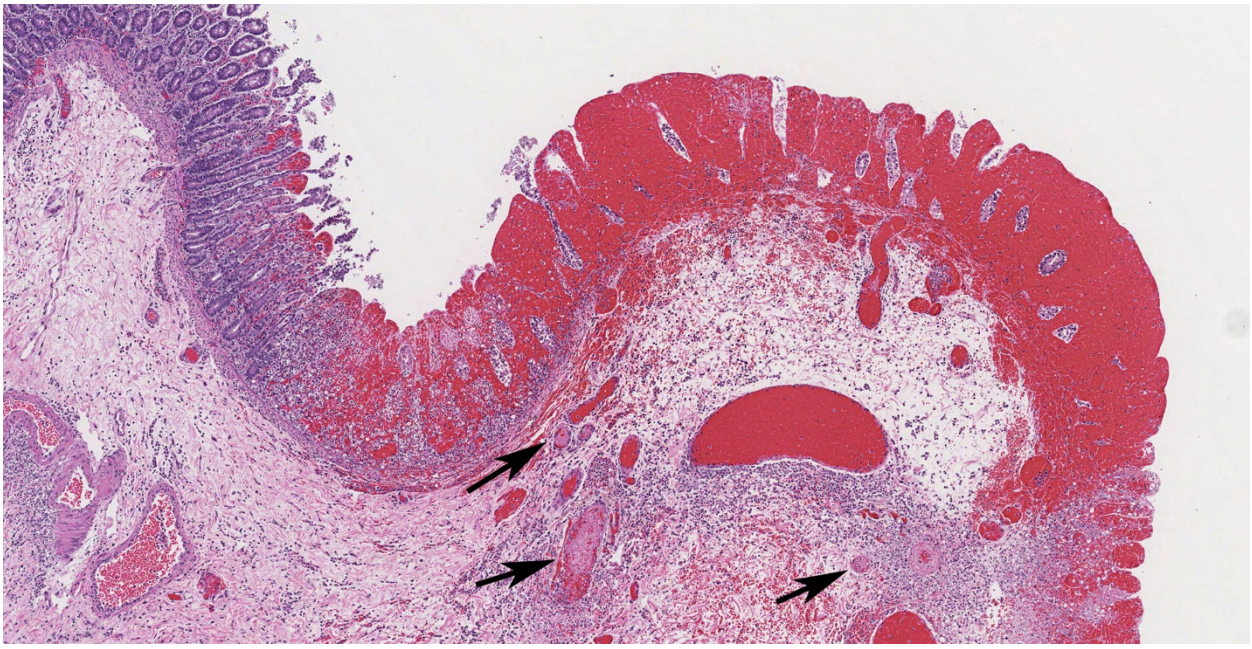
Laboratory results:
N/A

Microscopic description:
Colon, two sections:

The colonic wall is diffusely and markedly expanded by edema, multifocal hemorrhages and fibrin, and there are several focally extensive areas of mucosal hemorrhage. There are multifocal areas of hemorrhage in which there is a loss of differential staining of the mucosa with retention of tissue architecture (infarcts) and underlying mural blood vessels often contain fibrin thrombi. At higher magnification, there is a transmural inflammatory infiltrate composed primarily of neutrophils, with fewer lymphocytes, plasma cells and macrophages.



Colon, rhesus macaque. Two sections of colon are submitted for examination. At subgross magnification, there is diffuse severe submucosal edema and segmental hemorrhage. (HE, 5X)



Colon, rhesus macaque. There are numerous segmental areas of hemorrhage and necrosis. Within the edematous submucosa, there are numerous thrombosed and inflamed vessels (arrows). (HE, 53X)

Multifocally, within the apical colonic crypt openings and often extending into the lamina propria, there are mats of fungal hyphae admixed with yeast-like blastospores and blastoconidia arranged in short chains (pseudohyphae). Hyphae are 3-6 µm wide, septate and have parallel walls. Blastospores are 2-6 µm in diameter with occasional budding. The yeast forms are stained black on a Grocott's Methenamine silver stain.

Multifocally and frequently within the interstitial layers of the tunica muscularis, there are high numbers of protozoa with a distinct absence of a surrounding inflammatory reaction. These protozoa are ovoid or occasionally piriform and measure 6-12 µm in diameter with a single basophilic nucleus and are stained magenta on a Periodic acid-Schiff stain.

There is a lack of gut-associated lymphoid tissue (lymphoid depletion) and multifocally within the submucosa and lamina propria there is scattered apoptotic cellular debris (lymphocytolysis). In infarcted areas, there are numerous small colonies of mixed bacteria (opportunistic commensal bacterial overgrowth).

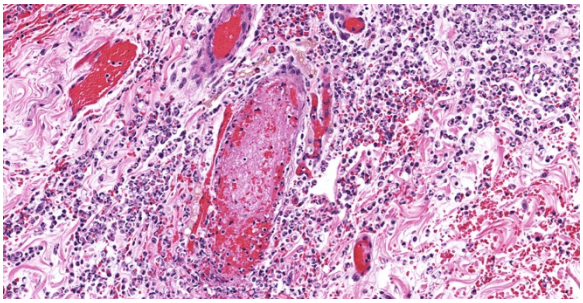
By immunohistochemistry, there is intracytoplasmic immunoreactivity to Ebola viral antigen within macrophages and spindle cells and within the lamina propria, there is immunoreactivity of extracellular material (fibrin and serum).

Contributor's morphologic diagnosis:

1. Colon: Infarcts, multifocal, with transmural edema, multifocal hemorrhage and intravascular fibrin thrombi.
2. Colon: Colitis, marked with mucosal fungal hyphae, blastospores and pseudohyphae (*Candida* species) and myriad protozoa.
3. Colon, gut-associated lymphoid tissue: Necrosis/apoptosis and depletion, diffuse, marked.

Contributor's comment:

Human viral hemorrhagic fevers (VHF) are typically caused by members of the four families: (1) *Arenaviridae* (several mammarenaviruses), (2) *Bunyaviridae* (several hantaviruses, nairoviruses, phleboviruses), (3) *Filoviridae* (certain ebolaviruses, marburgviruses) and (4) *Flaviviridae* (several flaviviruses *sensu stricto*).[9] VHFs are characterized by severe febrile illness with bleeding diathesis that culminated in shock and multiorgan failure. Key features in the



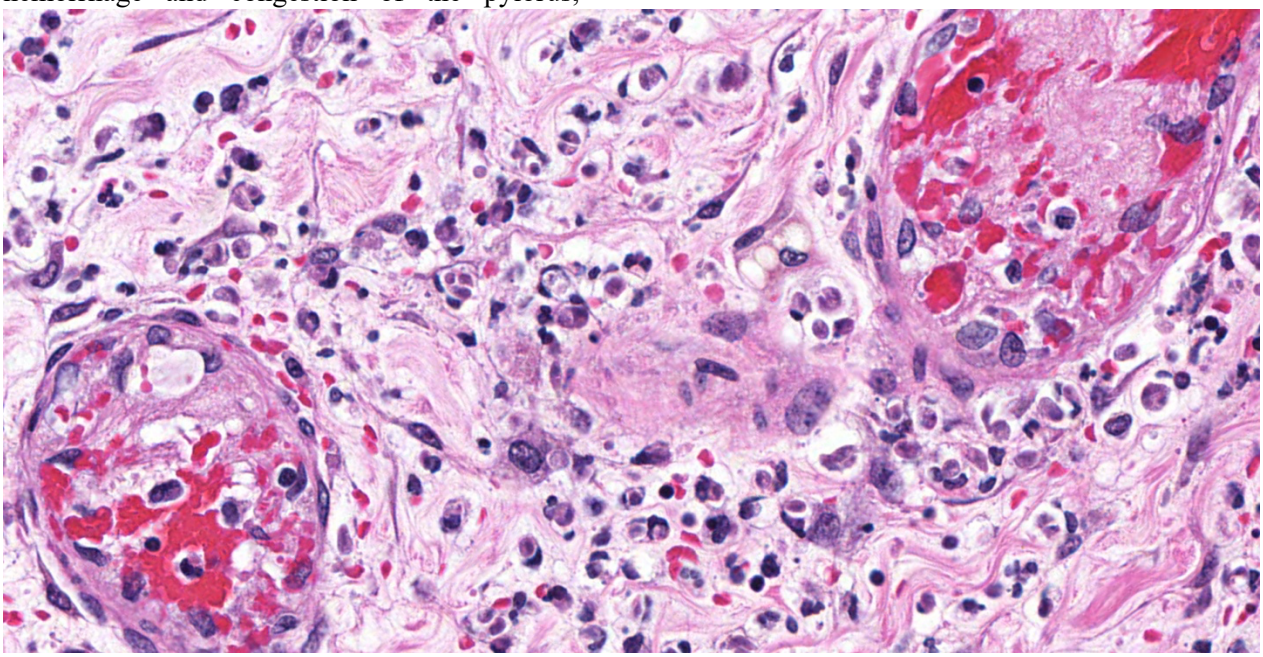
Colon, rhesus macaque. Higher magnification of thrombosed vessel subjacent to the area of hemorrhage (HE, 241X)

pathogenesis of VHFs include coagulopathy, tissue necrosis and immune suppression. These processes occur via activation of the coagulation cascade, massive release of cytokines and direct cellular lysis, most notably of lymphocytes.

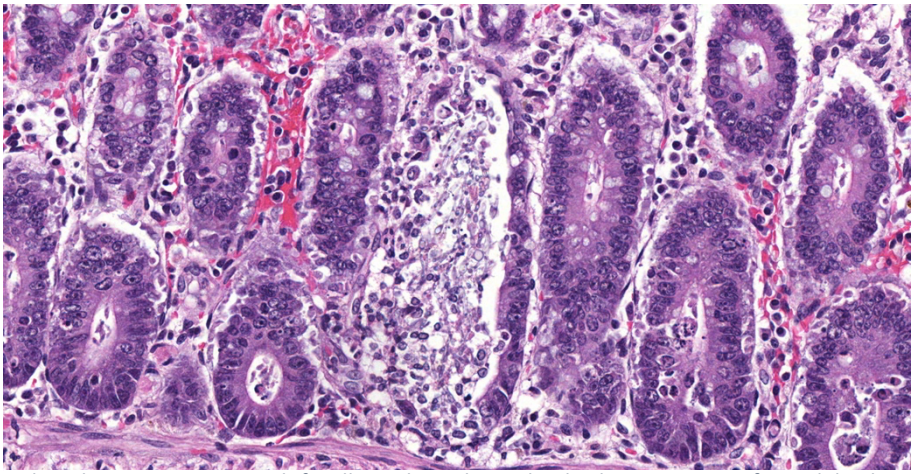
Filoviruses (family Filoviridae) include the Ebola virus (including Zaire, Sudan and Kikwit species/strains, among others) and Marburg virus genera and Reston virus which causes disease in nonhuman primates, but not in humans. The most consistent gross lesions in nonhuman primates experimentally infected with Ebola virus by either intramuscular or aerosol exposure include a red macular skin rash; a friable, pale liver; enlarged, firm spleen and lymph nodes; and hemorrhage and congestion of the pylorus, proximal duodenum and other segments of the gastrointestinal tract. Histologic lesions typically include hepatic necrosis and inflammation; lymphoid necrosis in the spleen, lymph nodes and other lymphoid tissue; fibrin that fills the splenic sinusoids; and intravascular fibrin thrombosis in the renal papilla, gastrointestinal tract and many other sites.[10,12] Other histologic findings in this animal included all of those listed above, as well as myocardial infarctions with myocardial necrosis.

Nonhuman primates infected with Ebola virus typically succumb to disease within 6-10 days following exposure. This animal lived slightly longer than average, perhaps due to supportive care; however, immune suppression in this animal was undoubtedly exacerbated by the administration of corticosteroids that allowed unchecked proliferation of commensal fungi and protozoa in the gastrointestinal tract. Corticosteroids, either endogenous or exogenous, can cause decreased immune responsiveness, suppression of inflammation and delayed wound healing.[2]

The histomorphologic and electron microscopic (EM) features of the colonic fungus are consistent with a species of *Candida*. *Candida* sp. are



Colon, rhesus macaque. In the vicinity of thrombosed vessels, numerous elliptical protozoa are present within the cytoplasm of macrophages and neutrophils. (HE, 600X)



Colon, rhesus macaque. Multifocally, within colonic glands, there are mats of pseudohyphae with blastoconidia and entrapped yeasts, consistent with Candida sp. (HE, 400X).

dimorphic fungi found in the environment, typically in the yeast form. After ingestion or inhalation, these yeasts can persistently reside on the mucosa of the gastrointestinal tract as a nonpathogenic commensal in immunocompetent animals. In the event of an immunocompromising event, these yeasts can shift to hyphal and pseudohyphal forms and proliferate on mucosal surfaces.[11] Typical manifestations of candidiasis in humans and animals, particularly young animals, occur on keratinized epithelial surfaces such as the skin, oral cavity, esophagus, rumen or vaginal mucosa. Systemic candidiasis can occur in any tissue, including the gastrointestinal tract, central nervous system and urinary systems.

Scanning and transmission EM was used to better characterize the protozoa. EM images identified the presence of 4-5 flagella protruding from the posterior pole, one of which is attached to the body wall forming an undulating membrane (image). The size, shape, presence of flagella and the single nucleus are consistent with a species of *Pentatrichomonas*.[4] Trichomonads are generally nonpathogenic commensal organisms found in many species of mammals and birds. As with other commensals, opportunistic infections can occur in cases of immune suppression or other disease conditions, and gastrointestinal trichomoniasis, specifically gastritis, has been reported in Rhesus macaques infected with simian immunodeficiency virus.[1,6]

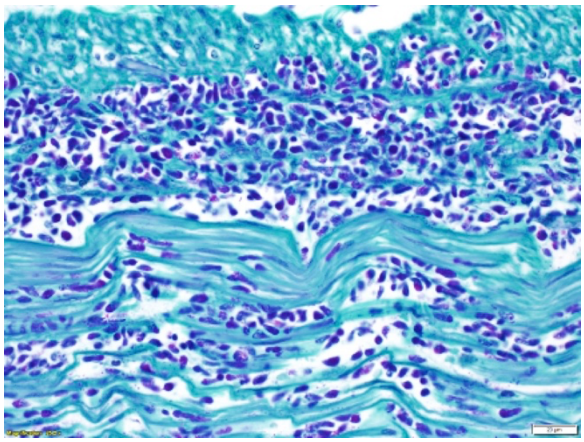
Contributing Institution:
United States Army Research Institute of Infectious Disease
Pathology Division
1425 Porter Street
Fort Detrick, MD 21702-5011

JPC diagnosis:

1. Colon, submucosal vessels: Vasculitis, necrotizing, multifocal, moderate with thrombosis, mucosal infarction, and severe submucosal edema.
2. Colon: Colitis, neutrophilic and histiocytic, multifocal and transmural, marked with intra- and extracellular trichomonads.
3. Colon: Colitis, neutrophilic and histiocytic, multifocal, moderate, with infiltrating yeasts and pseudohyphae.

JPC comment:

Ebola virus infection is a current and relevant topic of discussion, with periodic outbreaks most recently affecting African countries such as the Democratic Republic of the Congo, the Republic of South Sudan, the Republic of Uganda, Guinea, Liberia, and Sierra Leone.[3] As of 13 September 2020, the current epidemic (11th) of Ebola virus



Colon, rhesus macaque. Myriad ovoid to piriform extracellular and intrahistiocytic trichomonads expand the interstitial layers of the tunica muscularis. Periodic acid-Schiff. (Photo courtesy of United States Army Research Institute for Infectious Disease, Frederick, Maryland.)

(Zaire strain) disease in the Equateur Province of the Democratic Republic of the Congo has claimed the lives of 48 people, with 53 recovering. The approximately 50% mortality rate is common and may reach nearly 90% case fatality rates, and this particular province experienced a previous outbreak just two years prior. Resources for testing, medical providers, and medical supplies remains a challenge in these countries, with the World Health Organization contributing financially, and by providing healthcare workers.[7]

During the Ebola outbreaks among humans and great apes in Gabon and the Democratic Republic of the Congo from 2001 to 2003, numerous small vertebrates were captured and sampled in an effort to identify a natural host or reservoir host. The only animals implicated were a small number of fruit bats who either were serologic positives, or PCR positives with Ebola virus fragments present. However, no bats were serologic and PCR positive, and to date, there has been no successful virus isolation from a bat. Most evidence supports the hypothesis that bats are the likely natural host for Ebola virus, but they may also be incidentally infected by a different animal, the true natural host.[5] The book Spillover (2012) discusses considerations of emerging disease investigation, humanity's clash with nature, and the spillover of zoonotic diseases from wild animal populations into human populations.[8]

Ebola virus initially replicates in antigen-presenting cells, such as macrophages and dendritic cells, but may also infect numerous other cell types such as fibroblastic reticular cells, blood monocytes, hepatocytes, Kupffer cells, and cells of the adrenal gland and testicle. Peripheral mononuclear cells with ebolaviral infection fail to produce type 1 IFNs and inhibits IFN-α production through disruption of the RIG-1 pathway and Dicer-dependent protein kinase R. STAT proteins are also inhibited, blocking transcription of antiviral genes. In the later stages of disease, numerous cytokines (IL1β, IL-1RA, IL-6, IL-8, IL-10, IL-15, IL-16, TNF-α), chemokines, and growth factors (MIP-1α, MIP-1β, MCP-1, M-CSF, MIF, IP-10, GRO-α, and eotaxin) are increased from 5-1000 times normal, creating a cytokine storm resulting in organ failure, sepsis syndrome, and death.[3]

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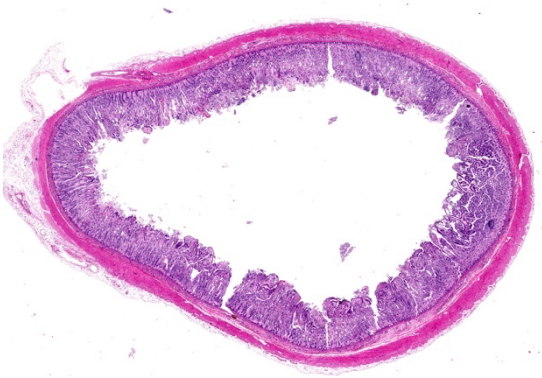


Jejunum, rhesus macaque. A section of jejunum is submitted for examination. Villar structure is not evident. (HE, 5X)

CASE 2: 14A030 (4048445-00)

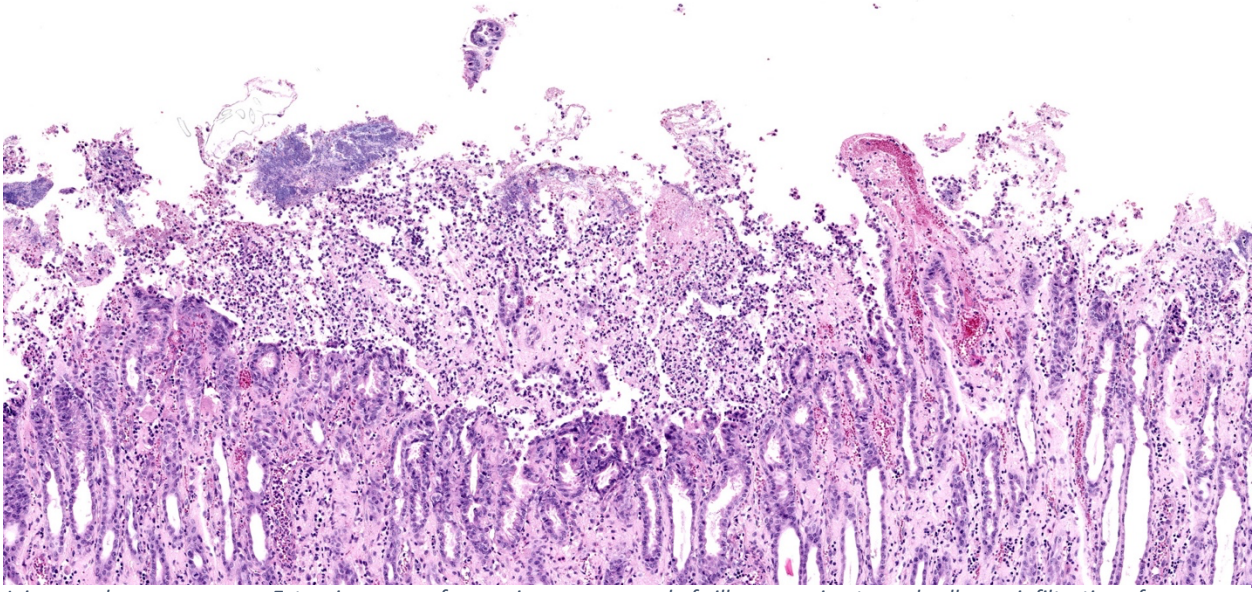
Signalment:
5.74-years-old, female Indian rhesus macaque (*Macaca mulatta*)

History:
This animal had chronic diarrhea. Three days prior to the necropsy, this animal presented with hypothermia, hypoglycemia, and dehydration. There was not improvement in spite of antibiotic treatment. Euthanasia was elected due to the poor prognosis.

Gross Pathology:
The animal showed thin body condition and muscular atrophy. Both eyes were sunken. The pancreas was pale and edematous. Diffusely the small intestine, cecum and colon were extremely dilated with large amount of fluid, blood-tinged fecal material. The mesenteric lymph nodes were edematous and enlarged.

Laboratory results:
Serum or EDTA blood testing for HVP2, MEASLES, SIV, SRV, STLV1, SRV2 were negative by MFIA and PCR. *Yersinia enterocolitica* were isolated from the colon 3 days prior to the necropsy.

Microscopic description:
Jejunum. The mucosa has multifocal to coalescing, mild to moderate erosions and/or ulcerations and replaced by large amount of eosinophilic cellular debris, degenerate neutrophils, fibrin and erythrocytes (hemorrhage). Admixed there are numerous colonies of 1 x 2 um bacilli (Gram negative). Diffusely the submucosa and serosa are mildly to moderately expanded by edema with many inflammatory infiltrates. Multifocally the blood and lymphatic vessels are dilated and surrounded



Jejunum, rhesus macaque. Extensive areas of necrosis are composed of villar necrosis, stromal collapse, infiltration of neutrophils, cellular debris, and a large colony of bacilli (upper left). (HE, 82X)

by edema and many inflammatory infiltrates (perivasculitis). Occasionally the tunica media of vessels is expanded and disrupted by inflammatory cells (vasculitis). Diffusely the lamina propria is expanded and contains abundant amorphous, finely fibrillar to waxy hyalinized deposits (Congo red stain positive).

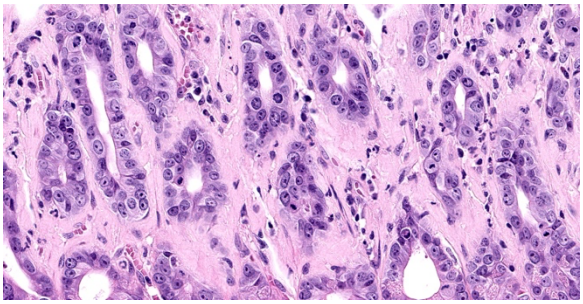
Contributor's morphologic diagnosis:

1. Small intestine: Enteritis, acute, diffuse, necrohemorrhagic, with perivasculitis, vasculitis, and colonies of bacilli, etiology consistent with *Yersinia enterocolitica*, Indian rhesus macaque (*Macaca mulatta*).
2. Small intestine: amyloidosis, diffuse, moderate, Indian rhesus macaque (*Macaca mulatta*).

Contributor's comment:

Yersinia is a member of *Enterobacteriaceae,* which encompasses 17 species including three important pathogenic species: *Y. enterocolitica, Y. pseudotuberculosis* and *Y. pestis. Y. enterocolitica* is a globally distributed gastrointestinal pathogen and usually causes a self-limiting acute infection beginning in the intestine and spreading to the mesenteric lymph nodes.[7] However, it can cause serious or chronic infections, particularly in immunocompromised individuals. The pathogenesis of *Y. enterocolitica* is not completely clear. *Y. enterocolitica* contains more than 16 virulence-associated genes. The plasmid gene of pYV/pCD and chromosomal genes of ystA, ystB, and ystC are the most important for the pathogenesis. pYV/pCD gene functions to allow bacterium to penetrate the intestinal wall. ystA, ystB, and ystC are the stable heat-stable toxin genes that may contribute to the pathogenesis of diarrhea.[8]

Y. enterocolitica infects many species of NHPs and causes outbreaks of diarrhea. Fatal yersiniosis has been documented in captive squirrel monkeys and gibbons. The major lesions are necrotizing enteritis and abscesses in many other organs.[7] The differential diagnosis in nonhuman primates includes *Shigella*, C*ampylobacter*, *Salmonella* and *E. coli*.



Jejunum, rhesus macaque. The lamina propria is expanded by abundant amyloid which compresses glands. There are increased numbers of mitotic figures within the crypts. (HE 398X)

Reactive amyloidosis is not an uncommon finding in rhesus in our colonies. The major organs involved are intestine, mesenteric lymph nodes, spleen, and liver, but less often in kidney and other organs. Reactive amyloidosis is caused by the deposition of serum amyloid, which is a circulating acute-phase reactant produced mainly by hepatocytes, and regulated by interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF). Reactive amyloidosis has been reported in human patients with rheumatoid arthritis and other chronic inflammatory diseases.[9] *Y. pseudotuberculosis* has been reported to be the cause of amyloidosis in humans,[1] but the association of *Y. enterocolitica* and entero-amyloidosis in this case is unclear.

Contributing Institution:

Department of Comparative Pathology
Tulane National Primate Research Center
http://tulane.edu/tnprc/

JPC diagnosis:

1. Small intestine: Enteritis, necrosuppurative, multifocal, moderate, with large colonies of bacilli.
2. Jejunum, mucosa: Amyloidosis, diffuse, moderate.

JPC comment:

Pigs are generally considered the reservoir host of *Yersinia enterocolitica*, and is a widespread pathogen across Europe and Poland. While many cases continue to be reported, detection and identification of this bacterium remain difficult. It has recently been shown that diagnostics can be improved with the use of Matrix-Assisted Laser

Desorption Ionization-Time of Flight Mass Spectrometry (MALDI TOF MS), but at the cost of time and additional media and equipment preparation.[6]

There are a number of virulence factors that aid *Y. enterocolitica* in establishing infection in tissues. Some of the most well characterized include the three adhesins/invasins Inv, YadA, and Ail, which facilitate attachment to host cells, and confer the ability to survive neutrophils and in Peyer's patches. Before attaching to target host cells, this bacterium has a flagella and motility increases its virulence. Either the *flhDC* or *fliA* gene must be functional to maintain a functional flagellum, and experimental inactivation results in decreased virulence *in vitro*. Lipopolysaccharide of the outer membrane is a potent virulence factor that is shared by Gram negative bacteria.[4]

However, the most important virulence factor is the Yop virulon, which elaborates its effectors to the extracellular space, the plasma membrane, and the host cell cytosol, and the Ysc type 3 secretion system (T3SS). The T3SS consists of the injectisome and the translocators YopB, YopD, and LcrV, and the T3SS counteracts multiple innate defenses of phagocytes by inhibiting cytoskeleton movement and preventing its phagocytosis.[4]

While more strictly public health facing, there has been research into the use of bacteriophages to limit human exposure and consumption of food contaminated by *Yersinia enterocolitica*. Viruses of *Podoviridae* and *Myoviridae* were shown to be safe in mice, and several phages significantly reduced the population of experimentally placed Y. enterocolitica on kitchen utensils and food. The bacteria exhibit different morphologic characteristics based on temperature, so whether this represents a potential *in vivo* therapy will require additional research.[5]

There is widespread concern about the prudent use of antibiotics in the treatment of susceptible disease, and research regarding alternative therapies against *Y. enterocolitica* continue. Aporphinoids are a subgroup of benzylisoquinoline alkaloid compounds found in plants, and several demonstrated significant antibacterial properties against Y. enterocolitica (as well as some efficacy against a *Salmonella enterica* strain, *E. coli* strain, and *Staphylococcus aureus* strain).[3] Additionally, colicin F_Y, which is typically produced by *Yersinia frederiksenii*, has demonstrated efficacy against *Y. enterocolitica* in mouse models. Murine *E. coli* isolates were transformed with a colicinogenic plasmid in order to induce production of colicin F_Y and tested *in vitro* and *in vivo* with excellent results.[2] Whether either of these represent potential future therapies will be determined by future research.

References:

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CASE 3: 16-A817 (4118327-00)

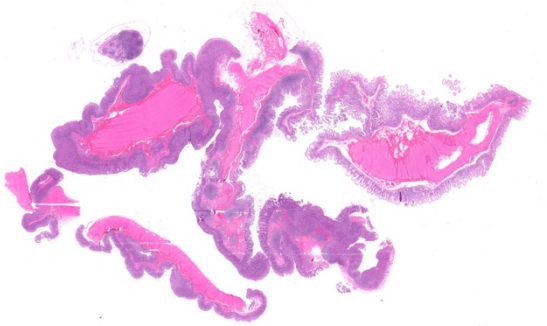
Signalment:
Juvenile (2.5-year-old) female Indian-origin rhesus macaque (*Macaca mulatta)*

History:
The animal was a colony assigned animal, born at the ONPRC and outdoor-housed in a social group. She presented for diarrhea and dehydration with 17% weight loss since the last recorded weight five months prior. There were no previous clinical presentations. Fecal cultures were positive for both *Campylobacter coli* and *Shigella flexneri*. Abdominal ultrasound revealed thickened stomach and small intestinal walls, gastric distension with hypoechoic ingesta and gas, and small intestinal hypermotility and distension with anechoic liquid digesta. Colonic wall thickness could not be measured; however, prominent layering with gas and liquid feces were noted within the large intestine. The diarrhea continued despite three weeks of antibiotic administration (enrofloxacin and azithromycin) followed by another week of anti-inflammatory therapy (sulfasalazine and prednisone). Due to poor prognosis, euthanasia was elected, and the animal submitted for post-mortem examination.

Gross Pathology:
Chronic negative energy balance (BCS 1.5/5), thickened, corrugated cecal and colonic mucosa with trichuriasis, and renal cortical pallor consistent with acute tubular necrosis.

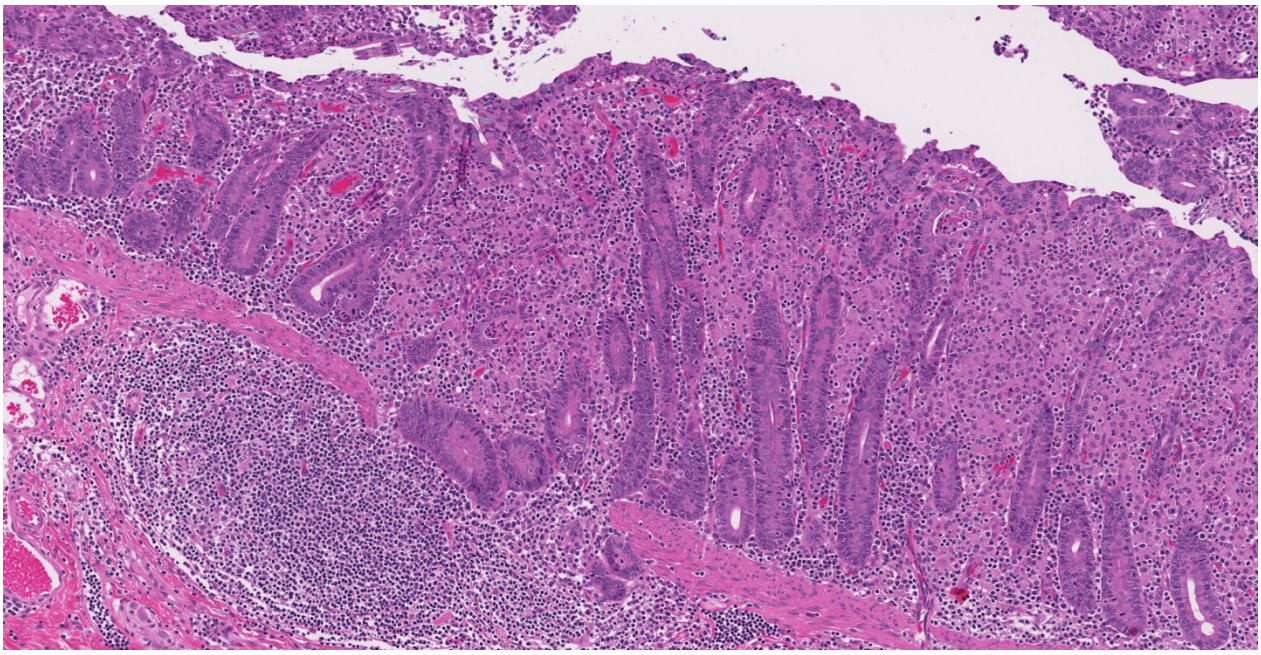
Laboratory results:
PCR of section of cecum were positive for *Mycobacterium avium*.

Microscopic description:
Ileocecal junction: Multifocally within the cecum are large aggregates of histiocytes with intracytoplasmic rod-shaped bacteria that expand



Gut, rhesus macaque. Multiple sections of gut, including ileum, cecum, and colon are submitted for examination, as well as mesenteric lymph node (upper left). (HE, 5X)

the lamina propria, separating and replacing crypts. The mucosa is diffusely thickened, up to 3x normal, with elongated crypts that are lined by epithelial cells with increased basophilia and mitoses (proliferation). The lamina propria is also expanded by increased lymphocytes and plasma cells, along with a moderate infiltrate of neutrophils and mucin-laden macrophages, which often extend into the muscularis mucosa and submucosa. Along the luminal surface is a paucity of normal bacteria (loss of commensal surface bacteria) and luminal mucosa is often attenuated with occasional loss of epithelial cells (erosion). Crypts are frequently dilated, lined by attenuated epithelium, and contain aggregates of degenerate neutrophils and karyorrhectic debris (crypt microabscesses). Hyperplastic gut-associated lymphoid tissue contains expanded germinal centers, along with occasional intact crypts (crypt herniation). There is focal hemorrhage within the cecal submucosa. Within the distal ileum, the villi are segmentally blunted (atrophy) and fused. The lamina propria is multifocally expanded by acellular, homogenous, eosinophilic material (amyloid). The subcapsular spaces and medullary sinus of the ileocolic lymph node contain a moderate infiltrate of foamy macrophages and rare multinucleated giant cells.



Colon, rhesus macaque. The lamina propria is expanded by large numbers of macrophages, admixed with numerous lymphocytes and plasma cells and rare eosinophils and neutrophils, which separate and often replace glands. Hyperplastic glands are often longer than normal, have a reduced number of goblet cells, and numerous mitotic figures.

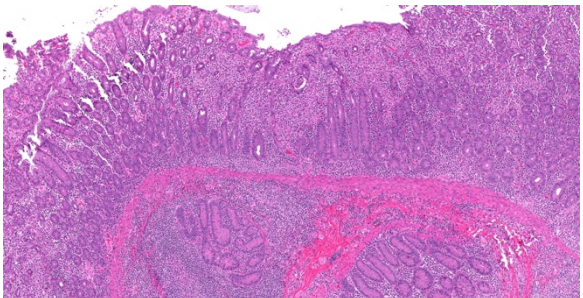
Contributor's morphologic diagnosis:

1. Cecum: Typhlitis, granulomatous, chronic, multifocal to coalescing, marked with histiocytic intracytoplasmic acid-fast rod-shaped bacteria
2. Cecum: Typhlitis, proliferative, lymphoplasmacytic, neutrophilic, chronic-active, diffuse, moderate to marked with crypt abscesses and herniation, multifocal mucosal erosion, loss of commensal surface bacteria, and focal submucosal hemorrhage
3. Ileum: Amyloid deposition, proprial, multifocal, mild with villus atrophy and fusion
4. Ileocolic lymph node: Histiocytosis, sinus and subcapsular, multifocal, mild with rare multinucleated giant cells

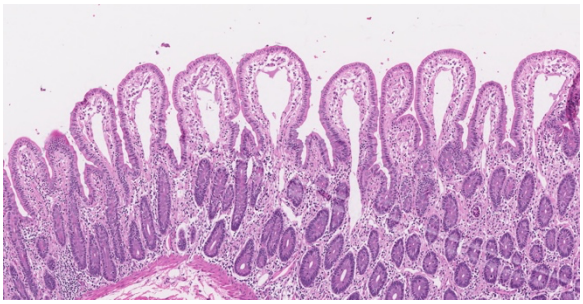
Contributor's comment:

Several disease processes are present in the section submitted that demonstrate classic enteric diseases of rhesus macaques: mycobacteriosis, chronic proliferative typhlocolitis, and secondary amyloidosis. Acid-fast histochemical stain revealed florid intracytoplasmic organisms within histiocytes of the cecum and colon. Microscopically, there were also small granulomatous nodules in the liver with rare intracytoplasmic acid-fast positive rod-shaped bacteria. PCR of paraffin-embedded sections of cecum confirmed *Mycobacterium avium* complex (*Mycobacterium avium-intracellulare*). Congo red histochemical stains highlighted congophilic material that demonstrated apple-green birefringence upon polarized light within the ileum, as well as in the stomach, jejunum, and colon.

Mycobacterium avium complex bacteria are ubiquitous in soil, dust, and aquatic



Colon, rhesus macaque. Other changes seen in the colonic mucosa include crypt abscesses (top center), and prolapse of hyperplastic glands into the underlying Peyer's patches (HE, 80X)

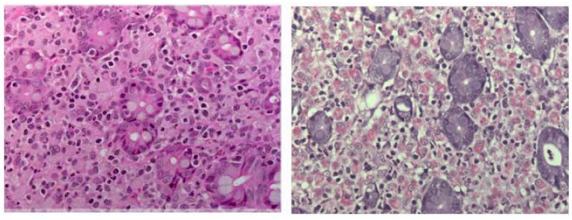


Ileum, rhesus macaque. There is marked edema in some segments of the ileum, resulting in marked expansion of villi and lacteal dilation. (HE, 200X)

environments, where mammals can be exposed to the organisms and develop subsequent disease.[8] Like *Mycobacterium tuberculosis*, mycobacteriosis can cause significant disease in old and new world nonhuman primates and occurs most commonly in macaques that are immune compromised.[7] As such, animals with mycobacteriosis are usually indoor-housed, protocol-assigned, and undergoing irradiation therapies or experimental infection with immunosuppression-causing pathogens such as simian immunodeficiency virus. In natural infections, mycobacteriosis is most commonly described in the small intestine, similar to Johne's disease (*Mycobacterium avium paratuberculosis*) in other veterinary species. However, disseminated disease does occur and may affect the colon, liver, and lymph nodes.[7] In the present case, the organisms were present in the large intestine and liver, but no small intestinal granulomatous inflammation was noted. Another unusual aspect of the case is that the animal was outdoor-housed with no known disease that could account for a state of immunosuppression.

Proliferative typhlocolitis, or chronic colitis of macaques, is the most common disease causing morbidity and mortality in many nonhuman primate facilities.[4] It is characterized by hyperplastic cecal and colonic mucosa with variable proprial infiltrates of lymphocytes, plasma cells, histiocytes, neutrophils, and eosinophils, crypt abscesses, loss of goblet cells, gut-associated lymphoid hyperplasia, and crypt herniation.[4] Other findings include superficial epithelial tags, presence of mitotic figures in the upper half of glands, and lack of filamentous bacteria that normally colonize the luminal surface. This entity occurs in both nursery-raised and group-housed, maternally reared animals. It is primarily seen in young animals between 1 and 3 years of age. Initial episodes of diarrhea may be associated with positive fecal cultures for *Campylobacter* spp[5] or *Shigella flexneri*, as in this case. Subsequent episodes are characterized by normal enteric flora and the variable presence of protozoa. The pathogenesis is thought to be complex involving repeated enteric infections, malnutrition associated with enteric disease, compromised mucosal defenses, environmental stresses, and possible hypersensitivity to dietary antigens.[4] Proliferative typhlocolitis is often seen in conjunction with acute renal tubular degeneration and necrosis, which is due to hypoperfusion caused by diarrhea-induced hypovolemia.

Secondary amyloid deposition in macaques is commonly associated with proliferative typhlocolitis due to the state of chronic inflammation. The other main condition associated with amyloidosis in macaques is chronic arthritis.[1] Microscopically, amyloid may be noted in the proprial tissues and around blood vessels of the gastrointestinal tract, interstitium of the renal medulla, spaces of Disse in the liver, cortical-medullary junction of adrenal glands, spleen, and within lymph nodes.[1] As in humans and other animals with chronic inflammation, secondary amyloid deposition in macaques is due to misfolded serum amyloid A (SAA) proteins.[2] Misfolded proteins undergo limited proteolysis before monomers assemble to form beta-sheets that make up the AA fibrils.[2] AA amyloidosis is considered to be species-specific and is typically non-transmissible between species.[6] New studies



Colon, rhesus macaque. Granulomatous inflammation in the cecum (left) with intrahistiocytic cytoplasmic acid-fast rod-shaped organisms (right). (Ziehl-Nielsen, 400X). (Photo courtesy of: Oregon National Primate Research Center, Oregon Health & Science University, 505 NW 185th Ave., Beaverton, OR 97006, http://www.ohsu.edu/xd/research/centers-institutes/onprc/)

in mice, however, are challenging this concept and have shown limited AA amyloid deposition with transfer of bovine and feline AA amyloid extracts in IL-1 receptor antagonist knock out mice.[9] In rhesus macaques, AA amyloid deposition in the intestine may be associated with chronic negative energy balance due to malabsorption, often compounding the clinical signs of chronic diarrhea.[1]

Contributing Institution:
Oregon National Primate Research Center
Oregon Health & Science University
505 NW 185th Ave.
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http://www.ohsu.edu/xd/research/centers-institutes/onprc/

JPC diagnosis:

1. Cecum and colon: Typhlocolitis, lymphohistocytic and proliferative, chronic, diffuse, severe with crypt abscesses and edema.
2. Ileum: Ileitis, lymphohistiocytic, diffuse, moderate with mucosal edema.
3. Ileum, mucosa: Amyloidosis, multifocal to coalescing, moderate.
4. Mesenteric lymph node: Reactive hyperplasia, with medullary histiocytosis.

JPC comment:
The contributor succinctly describes a case of multiple pathologies in this nonhuman primate. While proliferative colitis (or typhlocolitis) has been noted in colonies of macaques, there is potential overlap and causation with the condition Idiopathic Chronic Diarrhea ICD), which has been well characterized in populations at the California National Primate Research Center (CNPRC). In these cases, there is a lymphoplasmacytic colitis similar to that seen in human disease, including the subet of diarrhea-predominant irritable bowel syndrome (I-IBS). In the population of rhesus macaques analyzed, most had historic *Campylobacter* spp and/or trichomonad infections, and the ICD animals had positive culture results at much higher rates than the control animals. The ICD animals also had an absence of normal colonic bacteria of epithelium-oriented bacteria and an overgrowth of trichomonads, determined to be *Helicobacter macacae* and *Pentatrichomonas hominis* as compared to controls, with most having received antibiotic therapy. ICD animals also have decreased IL-4 expression in the ileum and colon, potentially suggesting either a preexisting condition that prevented development of these CD4+ Th2 cells, or there was a disease induced shift away from this expression pattern. While ICD may be related to the described proliferative typhlocolitis and shares several features, additional research is needed to elucidate the causal relationships between gastrointestinal flora, disease, and any preexisting conditions.[3]

As the contributor stated, most immunocompetent macaques do not experience significant pathology from *Mycobacterium intracellulare*. However, there have been reports of intestinal pathology associated with *M. intracellulare*, which included mild to severe thickening of the intestinal, cecum, or colon wall, thickening of the intestinal mucosa with a granular to corrugated appearance, with prominent lymphatic channels in the mesentery. Typically, the inflammation is of a granulomatous character, with acid-fast bacilli visualized in macrophages with Ziehl-Neelsen stained sections. In this case, it becomes difficult to determine whether 1) an immune suppressing condition caused the susceptibility to *M. intracellulare*, which resulted in the eventual mucosal changes seen consistent with proliferative typhlocolitis, or 2) the course of chronic typhlocolitis resulted in sufficient immune suppression that the animal experienced pathologic effects from *M. intracellulare*.[7] This animal may have been infected with various immune suppressive viruses as well, such as simian retrovirus (SRV), of which there are several potential strains (SRV-1, SRV-2, SRV-3, SRV-5).[7] A more distant possibility remains infections with simian immunodeficiency virus, though there is was no exposure noted, and no histopathology consistence with that disease reported.

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CASE 4: 16154 (4117663-00)

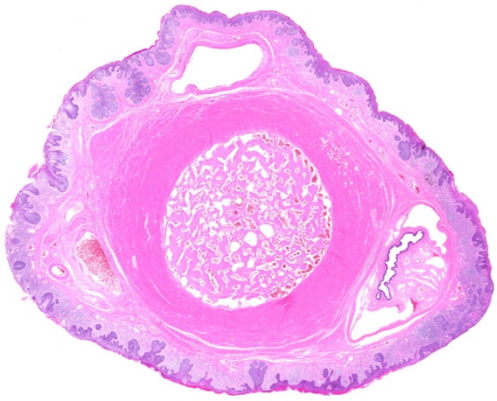
Signalment:
Seventeen-year-old, male, Chinese-born rhesus macaque (*Macaca mulatta*) with an unknown breeding history.

History:
This animal was a control animal and blood donor.

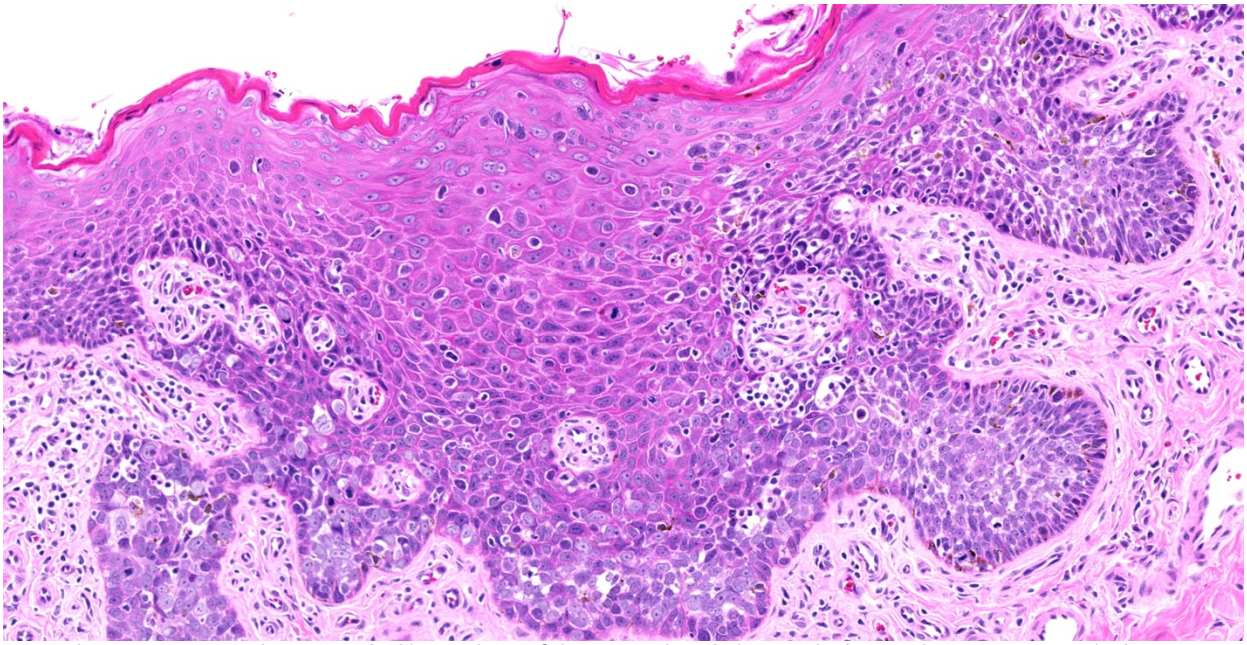
Gross Pathology:
N/A

Laboratory results:
N/A

Microscopic description:
Diffuse hyperplasia of the stratum basale is present in the skin of the penis, in many places forming downward projections which extend into the dermis. In one area, the epithelium is disorganized with loss of polarity and haphazardly arranged, enlarged polygonal cells which vary in size up to 35µm in diameter). These cells have distinct borders, moderate amounts of amphophilic cytoplasm, large vesicular nuclei, and single prominent nucleoli. Mitoses are abundant, at up to 22 per ten 40x fields. Ventral to the urethra, the basement membrane is breached by small cords and clusters of neoplastic epithelial cells. In addition, erosions and ulcers are present, the dermis is infiltrated by lymphocytes, plasma cells and fewer neutrophils, and aggregates of brownish-black pigment are present in the dermis. Clusters of prominent thick-walled capillaries lie beneath an area of ulceration. A keratin pearl is surrounded by neoplastic cells and rare individual cell keratinization (dyskeratosis) is present in the epidermis.



Penis, rhesus macaque. A cross section of the penis is submitted. At low magnification, there is marked mucosal hyperplasia with formation of arborizing rete ridges as well as submucosal inflammation. (HE, 5X).



Penis, rhesus macaque. There is marked hyperplasia of the mucosal epithelium with abnormal maturation, marked expansion of the basal layer and mitotic activity at numerous layers (dysplasia). (HE, 200)

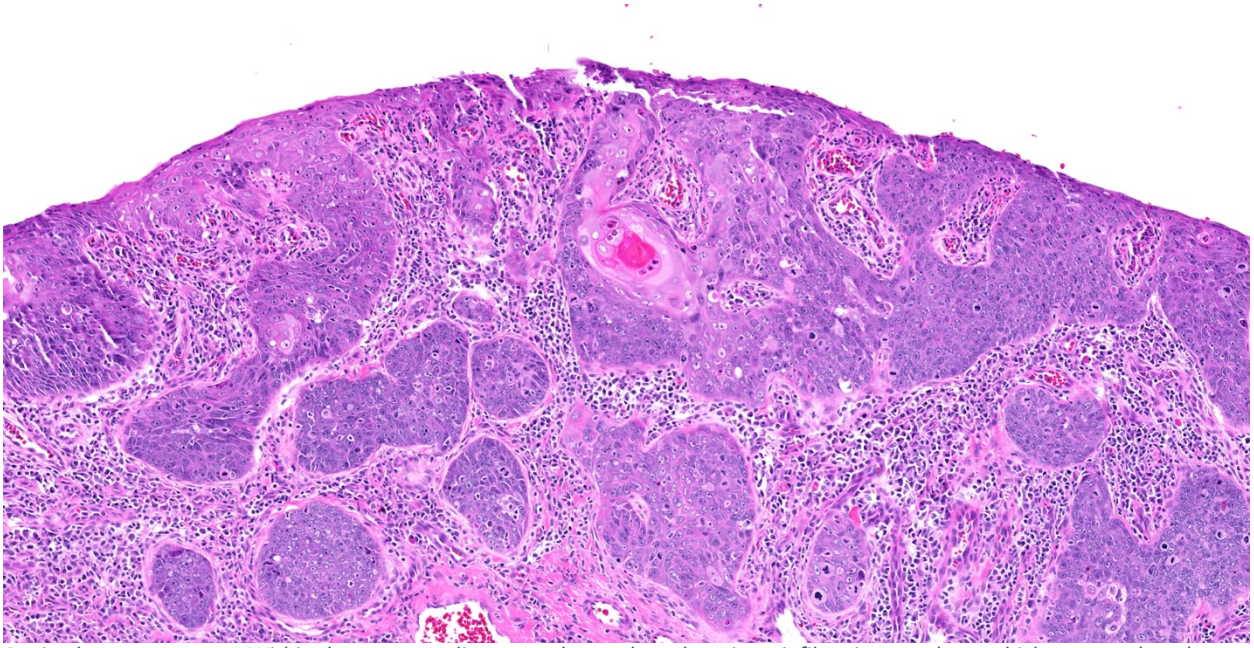
Contributor's morphologic diagnosis:
Epithelial hyperplasia and dysplasia, focal, marked with ulceration, moderate chronic lymphoplasmacytic dermatitis, and focal microinvasive squamous cell carcinoma, penis

Contributor's comment:
Although the histologic findings were suggestive of papillomavirus-induced changes, the PCR from paraffin embedded sections for rhesus papillomavirus type D (RhPV-d) was negative. Immunohistochemical staining of these sections, however, was positive for markers of proliferation (p16 and Ki67) and dysregulation of the tumor suppressor p53. Possible interpretations for the negative PCR result include infection with a non-RhPV-d strain, absence or low levels of virus in sectioned tissues, or the lesions were not of viral etiology.

Papillomaviruses are small, non-enveloped, double-stranded DNA viruses of the family Papillomaviridae which infect epithelial cells. A variety of human papillomaviruses (HPV) are associated with anogenital and oropharyngeal neoplasia.[5] Out of the 100+ HPV types, high risk HPVs (HPV 16, 18, 35 and 41) are capable of inducing malignant changes in the human reproductive tract. Penile carcinoma is less well-characterized compared to female reproductive tract carcinomas associated with HPV[9], and only thirty percent of penile cancers are associated with HPV.[1]

The expression of p16 and p53 are associated with HPV-HR (high risk)-induced penile carcinogenesis while p16 negative, p53 positive lesions were identified in HPV-negative cancers.[11] In another human study, p16 and high Ki-67 expression were considered efficient diagnostic markers for precancerous lesions in HPV positive individuals.[10]

In non-human primates, thirteen identified RhPVs belong to the alpha-PV group, and most are unrelated to HPV.[3] However, RhPV-1, was found to be closely related to an HPV16 which was isolated from an inguinal lymph node in a rhesus macaque with a penile squamous cell carcinoma.[8,12] Cervical intra-epithelial neoplasia in female cynomolgus macaques is associated with the most common type of PV, RhPV-d, which is closely related to RhPV-1 and HPV16 based upon L1 and E6 ORF sequences. RhPV-d is transmissible between macaques, as the viral gene product, E1^E4, was confirmed in two RhPV-d donors and three recipients in cervical



Penis, rhesus macaque. Within the mucosa adjacent to the urethra, there is an infiltrative neoplasm which nests and cords neoplastic epithelial cells breach the basement membrane. There is central keratinization of one cord (upper center). (HE, 120X)

cells.[15] The initial report of RhPV-1 was from a penile squamous cell carcinoma in a male rhesus macaque,[12] but other reports in non-human primates are nonexistent.

Non-HPV premalignant penile lesions in humans are primarily associated with lichen sclerosus which has a predilection for the genital area.[7] Penile intraepithelial neoplasia (PeIN) is classified into differentiated PeIN, which are most commonly associated with chronic inflammation, and undifferentiated subtypes (warty, basaloid and mixed warty-basaloid) that are strongly associated with HPV.[13] PeINs have also been classified based upon p16/p53/Ki-67 immunohistochemical panels. Differentiated PeIN is typically p16 negative and Ki-67 positive and has variable p53 expression. However, p16 and Ki-67 staining are consistently positive in basaloid and warty PeINs, again with variable p53 positivity.[4]

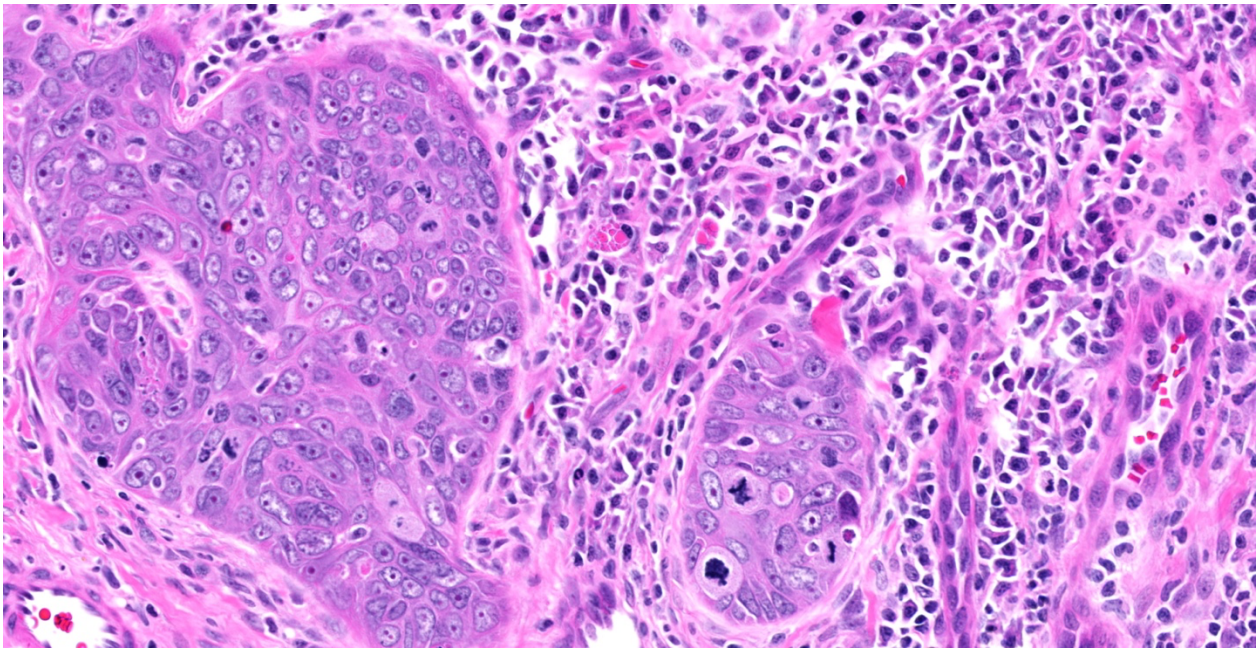
Contributing Institution:
http://www.wakehealth.edu/Comparative-Medicine/

JPC diagnosis:

1. Penis: Squamous cell carcinoma.
2. Penis, mucosa: Hyperplasia, circumferential, severe, with dysplasia, and moderate lymphoplasmacytic submucosal inflammation.

JPC comment:
The contributor summarizes this case succinctly. The use of the descriptor microinvasive is used much more commonly in human pathology than in veterinary pathology. While most commonly applied to squamous cell carcinoma in the larynx or the cervix, it has been applied to SCC in other locations as well. The definition varies, but most often includes:

1. The presence of scattered malignant cells just below the basement membrane
2. The presence of malignant cells with no invasion beyond 2mm
3. The presence of malignant cells within 1-2mm of the basement membrane without angioinvasion
4. The presence of discrete foci of malignant epithelium invading through the basement membrane



Penis, rhesus macaque. There is marked anisocytosis and anisokaryosis of infiltrating cells and mitoses are frequent and occasionally bizarre. (HE, 400X)

However, a simpler summarization is an invasive carcinoma extending into the stroma not more than 0.5mm beyond the basement membrane and with no evidence of angioinvasion.[14]

Papillomaviruses have been associated with penile lesions in other species as well. Young bulls commonly develop fibropapillomas on the head of the penis as a result of infection with bovine papillomavirus 1. While fibropapillomas are benign, *Equus caballus* papillomavirus 2 is implicated in the malignant transformation to SCC in the penis of the middle-aged horse.[6] Similar to this case, p53 is most often overexpressed, and in situ hybridization (ISH) has confirmed E6/E7 viral nucleic acids in neoplastic cells.[15]

Using Ki67 as a marker of proliferation has been well established in human pathology and is most well characterized in mast cell tumors in veterinary literature. Ki67 is a non-histone nuclear matrix protein expressed in all phases of the cell cycle, except G0. It has a relatively short half-life of 1 hour, and an increased from the baseline proliferative index may indicate neoplasia. In human penis SCC research, a statistically significant threshold of <20% Ki67 immunolabeling correlated with Grade 1 neoplasms, up to 46% for Grade 2, and Grade 3 neoplasms had up to 66% of neoplastic cells immunolabeled. These determinations are useful when attempting to correlate proliferation index with grade and prognosis. While this data may not translate exactly to nonhuman primates, it may serve as a starting point for creating classifications for SCC in nonhuman primates.[2]

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