Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE
2020-2021

Conference 5

23 September, 2020

Joint Pathology Center
Silver Spring, Maryland

CASE 1: S1808480 (4135076-00)

Signalment: A 12-week-old, female raccoon (*Procyon lotor*)

History: Raccoon presented with a history of progressive illness and diarrhea.

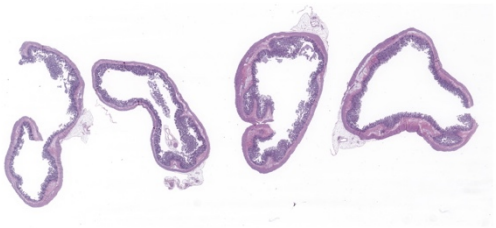
Gross Pathology:
The carcass was in fair nutritional condition, with a small amount of fat reserves, but still well fleshed. Bilaterally, the lungs were diffusely, moderately wet and had increased firmness, with multifocal to coalescing, whitish/cream-colored discoloration. The lumen of the distal portion of the trachea and main bronchi contained soft to friable, yellowish material. The perianal region and fur were matted with yellowish fecal material. Minimal contents were found in the small intestine, and the large intestine contained small amounts of yellowish-greenish pasty contents.

Laboratory results:
Cryptosporidium sp. was detected by PCR targeting the 18S rRNA gene on mucosal scrapes of the small intestine of this raccoon. The sequenced amplicon clustered with the *Cryptosporidium* skunk genotype. We also performed additional fecal analysis using special stains (modified acid fast) which showed rare numbers of *Cryptosporidium* spp. ELISA was performed on small intestine contents being also positive for *Cryptosporidium* spp. Transmission electron microscopy also demonstrated small rounded protozoa compatible with *Cryptosporidium* spp. No parasite eggs were detected in feces via fecal flotation.

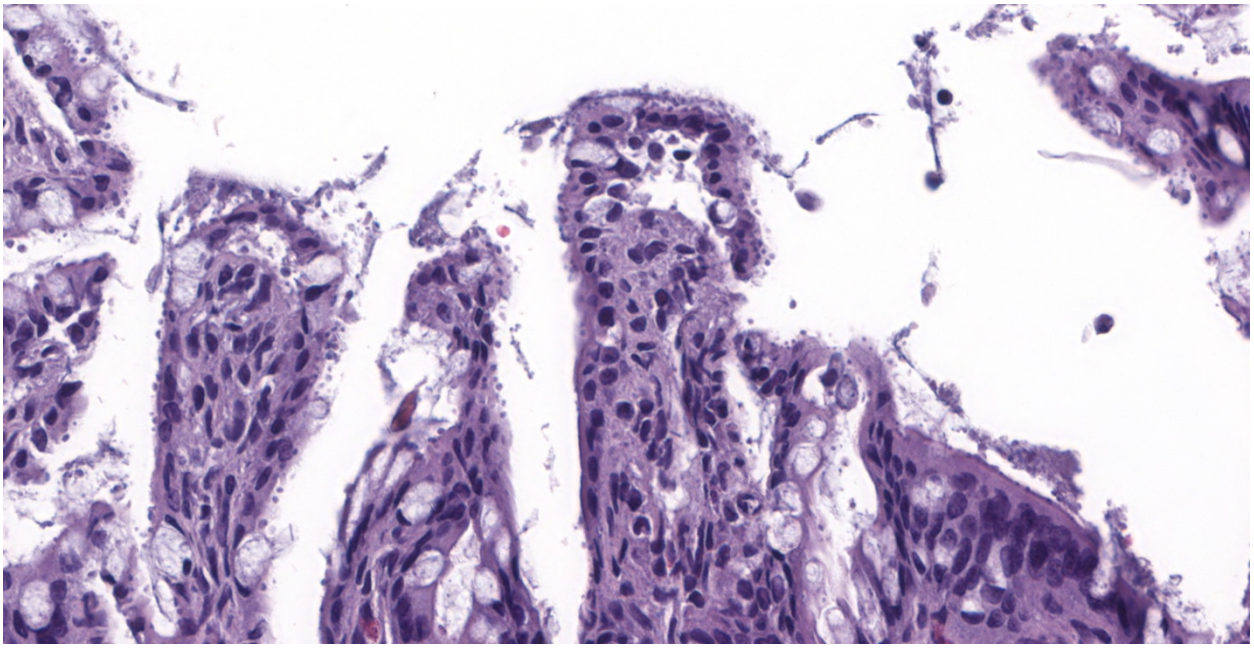
CDV immunohistochemistry in lung and intestinal tissue was positive. Canine parvovirus immunohistochemistry was negative on intestine tissue.

Additional tests performed on this case include a negative rabies test, and moderate numbers of *Escherichia coli* isolated from lung tissue.

Microscopic description:
There is diffuse atrophy of the intestinal mucosa (villus atrophy) and multifocal infiltration of



Intestine, raccoon. Four sections of mildly autolytic intestine are submitted for examination. Villi are markedly shortened in all sections. (HE, 5X)



Intestine, raccoon. Numerous Cryptosporidium sp. schizonts and gamonts line the atrophic villi. (HE, 400X)

eosinophils in the lamina propria. Lining the apical portions of the intestinal epithelium are multiple, 5-10 um round, heterogeneously basophilic structures (morphology compatible with *Cryptosporidium* sp.). There is multifocal necrosis of crypt epithelium, while in other areas crypts appear multifocally dilated and lined by attenuated epithelium. These dilated crypts often contain sloughed epithelial cells, cell debris, and a small number of neutrophils in crypt lumina.

Contributor's morphologic diagnosis:
Small intestine: Enteritis, necrotizing, with villus atrophy, crypt necrosis and intralesional cryptosporidia, raccoon (*Procyon lotor*)

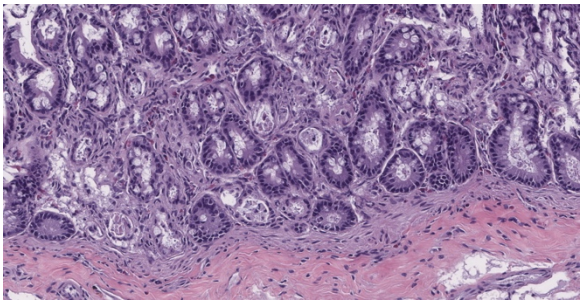
Other morphological diagnoses (not present in slide): Bronchointerstitial pneumonia, diffuse, marked, with intracytoplasmic and intranuclear inclusion bodies (compatible with infection by canine distemper virus); splenic lymphoid depletion, mild to moderate.

Contributor's comment:
Cryptosporidiosis is a protozoal disease of public health importance, caused by *Cryptosporidium* spp., a group of apicomplexan parasites that cause intestinal disease and are found in several vertebrate species including mammals, reptiles, birds and humans. Due to habitat encroachment from urbanization of forested land, wildlife species are coming into contact with human pathogens and becoming accidental carriers. Because of this, there is an interest in determining the carrier status of synantropic wildlife (species co-habiting in urbanized land), such as raccoons for human pathogens.

Raccoons may play a possible role in contaminating the environment, including urban areas and ecosystems, with pathogens of zoonotic relevance such as *Cryptosporidium* spp. and microsporidia (*Enterocytozoon* sp. and *Encephalitozoon* sp.), being either carried by these animals or acquired by interaction with other wildlife.

Cryptosporidium skunk genotype

Until recently, it was thought that *Cryptosporidium* spp. showed a strong host-adaptation and thus wildlife infections did not appear to pose a risk to human health. This thought becomes evident in the taxonomy of *Cryptosporidium* species, where many are still named after their host and are still referred to as 'host adapted'. Nevertheless, recent evidence suggests that some wildlife adapted genotypes are capable of inducing intestinal disease in humans,[17] which is a concern especially in areas



Intestine, raccoon. Multifocally, crypts are ectatic, lined by attenuated epithelium and contain degenerate epithelium admixed with cellular debris (crypt abscesses) (HE, 350X)

where numerous host species commingle and share resources which turn into common infection sources (e.g. contaminated water).

Multi-locus genetic characterization indicate that host adaptation is a general phenomenon of *Cryptosporidium* as specific genotypes tend to be detected in similar groups of animals.[24] These studies have also detected an extensive genetic diversity within *Cryptosporidium* which may suggest that host-parasite co-evolution may contribute to the genetic heterogeneity observed.[24] Currently, there are 20 valid species recognized for *Cryptosporidium*. The majority of human infections are caused by *Cryptosporidium hominis* or *Cryptosporidium parvum,* but *Cryptosporidium meleagridis*, *Cryptosporidium felis*, *Cryptosporidium canis*, *Cryptosporidium suis*, *Cryptosporidium muris*, *Cryptosporidium andersoni*, *Cryptosporidium hominis* monkey genotype, cervine genotype, and the chipmunk genotype I have also been detected.

Multiple studies in the early 2000s found that in Europe the *C. parvum* bovine genotype was the main cause of human infections instead of *C. parvum* human genotype.[2,10] The human genotype is still the main cause of infections in humans elsewhere in the world, but this finding justifies the investigation of other genotypes as causative agents of human diarrheal disease. Raccoons are infected by the *skunk genotype*, which was thought to only be found in wild or zoo animals.[21,24] The *skunk*, *rabbit* and *horse genotypes* were found in three separate cases of human diarrhea in the UK.[17] Other known hosts for the *Cryptosporidium skunk genotype* include the Eastern gray, American red and fox squirrels, river otters, and striped skunks in the USA.[22,25]

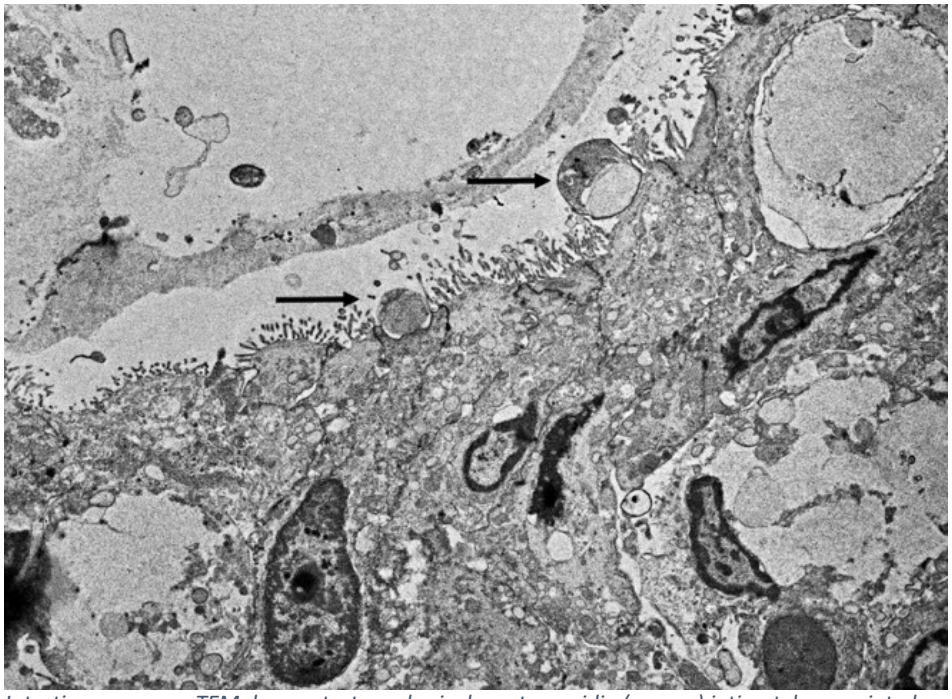
Distemper and raccoons

Distemper is caused by the canine distemper virus (CDV) of the genus Morbillivirus, family Paramyxoviridae, a virus spread by body excretions and secretions (urine), an important pathogen with a fatality rate only overtaken by rabies in domestic dogs.[23] CDV is capable of inducing virulent disease in a range of mammals, particularly in juveniles. If animals overcome the infection, they can develop lifelong immunity or die within a short time post-infection (20-120 days).[1] Natural and vaccine-induced infections have been reported in all families of terrestrial carnivores and also in free-ranging felids where the virus has recently caused large-scale epidemics.[3,19]

Clinical signs include diarrhea, dyspnea, neurological signs and profound immunosuppression.[4] There are regular epidemics in free-ranging raccoons in the U.S., indicating that the infection is endemic in some North American raccoon populations, playing a role in the distemper epidemiology in domestic dogs and non-domestic zoo populations.

As mammals, all procyonids are susceptible to natural CDV infections, resulting in multiple records of natural CDV infections in raccoons and vaccine-induced infections in kinkajous (*Potos flavus*).[6,11,12,13,18,20] Clinical presentation in procyonids is presumed to be similar to that of domestic dogs, including cystitis with pyuria,[16] as well as diarrhea, dyspnea, and profound immunosuppression.[4] Raccoons can develop neurological signs and become icteric.[12] It is possible that the profound immunosuppression caused by CDV makes raccoons (especially young individuals) vulnerable to be colonized by moderate to large numbers of cryptosporidia, and exacerbates gastrointestinal signs.

Changes to the crypts similar to those observed in this case are highly suggestive of canine parvovirus. However, testing for this virus was negative. It is possible that these changes were produced by *Cryptosporidium* infection as



Intestine, raccoon. TEM demonstrates spherical cryptosporidia (arrows) intimately associated (intracellular, extracytoplasmic) with enterocytes. (Image courtesy of : California Animal Health and Food Safety Laboratory, UC Davis, San Bernardino branch. https://cahfs.vetmed.ucdavis.edu/locations/san-bernardino-lab)

previously reported.[8] It is also possible that the crypt changes were associated with concomitant distemper virus infection.[7]

Contributing Institution:
California Animal Health and Food Safety Laboratory, UC Davis, San Bernardino branch.
https://cahfs.vetmed.ucdavis.edu/locations/san-bernardino-lab

JPC diagnosis:

1. Intestine: Villar blunting, diffuse, severe, with mild eosinophilic enteritis and numerous epithelial-associated apicomplexan schizonts and gamonts, raccoon, procyonid.
2. Intestine: Enteritis, necrotizing, multifocal, mild, with multifocal crypt abscesses.

JPC comment:
During the conference, there was debate about what cellular components are required to designate something a "crypt abscess." The literature varies between human pathology and veterinary pathology, and the standard terminology does not transfer between disciplines seamlessly. In human pathology, a neutrophilic component is required to be present to form a crypt abscess, while in veterinary pathology it may be composed of sloughed and necrotic cells without neutrophils. While the debate focused on terminology, the most important aspect of this topic is proper identification of neutrophils when present in order to more correctly identify the pathologic process.

Cryptosporidiosis has been documented in mammals, birds, reptiles, fish, and other species. In coccidia, the whole life cycle is completed in a single host. While the lifecycle and life stages help classify coccidia from other apicomplexans, there are ultrastructural features that provide more specific classification. Visible structures in apicomplexans include a conoid, rhoptries, and micronemes.[5]

In 1993, in Milwaukee, Michigan, spring snowmelt and rainwater raised the water level of the Menomonee and Kinnickinnic rivers as they fed the Milwaukee, ultimately dumping into Lake Michigan. The public drinking water of Milwaukee came from Lake Michigan and was processed according to federal water treatment guidelines and testing. Unfortunately, the largest concern of the time was bacterial pathogens, and large amounts of chlorine was assumed to be sufficient treatment. Unfortunately, the increased water flow had carried *Cryptosporidium* oocysts into Lake Michigan, through the treatment facilities, and to the faucets of residents. Ultimately, new tests using ELISA and fluorescent antibodies were used to find oocysts in the drinking water. Following the resolution of

this outbreak, it was estimated that approximately 400,000 people had fallen ill, with about 4000 hospitalized, and more than 100 people had died, making this the largest waterborne outbreak of disease in the United States at the time.[14]

In a study of *Cryptosporidium* outbreaks in human populations from 2009-2017, there were 444 outbreaks recorded, across 40 states and Puerto Rico. The two most implicated risks included exposure to treated recreational water (pools, water playgrounds, and similar) in 35% of cases, and contact with cattle in nearly 15% of cases.[9]

While apicomplexans cause significant disease in terrestrial animals most often, there are marine animals that exist with a more symbiotic relationship with these parasites. Research on the shipworm, a marine mollusk that burrows in and eats submerged wood, gastrointestinal tract revealed no microbial flora. Intracellular bacteria in the gills of the clam produce cellulolytic enzymes necessary for digestion of wood, but also plays a role in maintaining a sterile gut environment. A compound called tartrolon E (trtE) was isolated and found to have broad anti-apicomplexan activity *in vitro*, including *in vivo* efficacy against *Cryptosporidium parvum* in neonatal mice. This may represent the basis for a new therapy for this common disease across the world.[15]

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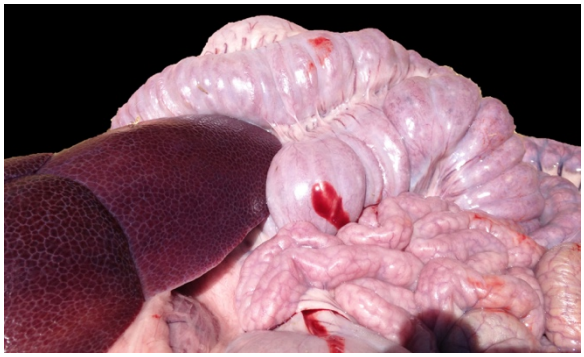
CASE 2: M17-18662 (4118631-00)

Signalment: 12-14 weeks old, unknown gender and breed, pig (*Sus scrofa domesticus*)

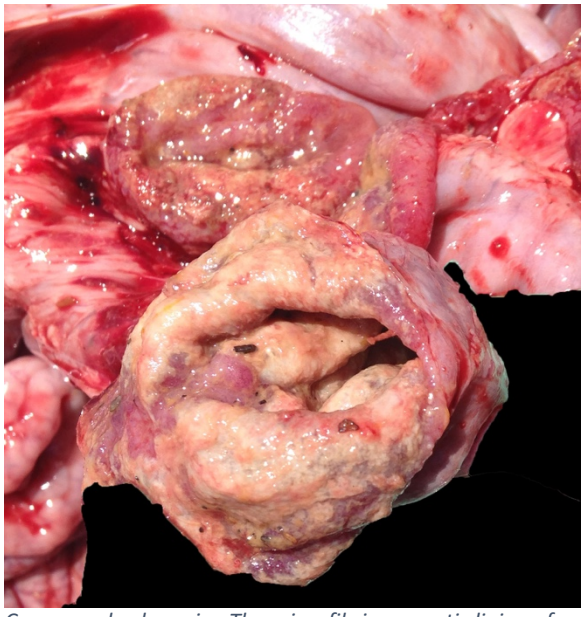
History:
This piggery had 33 breeding sows, several boars, including three introductions within the last 12 months, and home-bred piglets, weaners and growers. Under temporary new management approximately 50 weaners had died in the past 6 months, following intermittent bouts of inappetence, diarrhoea, lethargy and wasting. Sows were up to date with vaccinations for erysipelas, leptospirosis and parvovirus, and all animals were being fed age-appropriate commercial feed. The property had issues with rodent control and flooding in the weaner shed. Samples were submitted from weaners, including one dead (pig 1) and two euthanized (pigs 2 & 3), that had diarrhoeic faeces containing undigested feed, and were weak and wasted.

Gross Pathology:
The small and large intestines in all three weaners were grossly thickened and were lined by diphtheritic membranes in pig 3.

Laboratory results:
PCR of faeces (pigs 1 and 2) was positive for *Lawsonia intracellularis* (LI) and *Brachyspira pilosicoli*. Samples submitted between pigs 1 and 2 were selective salmonella culture was negative, PCR was negative for *Brachyspira hyodysenteriae,* porcine delta coronavirus



Cecum and colon, pig. There is segmental thickening of the large intestine. (Photo courtesy of: State Veterinary Diagnostic Laboratory (SVDL), Elizabeth Macarthur Agricultural Institute, New South Wales, Australia).



Cecum and colon, pig. There is a fibrinonecrotic lining of the affected segments of intestine. (Photo courtesy of: State Veterinary Diagnostic Laboratory (SVDL), Elizabeth Macarthur Agricultural Institute, New South Wales, Australia).

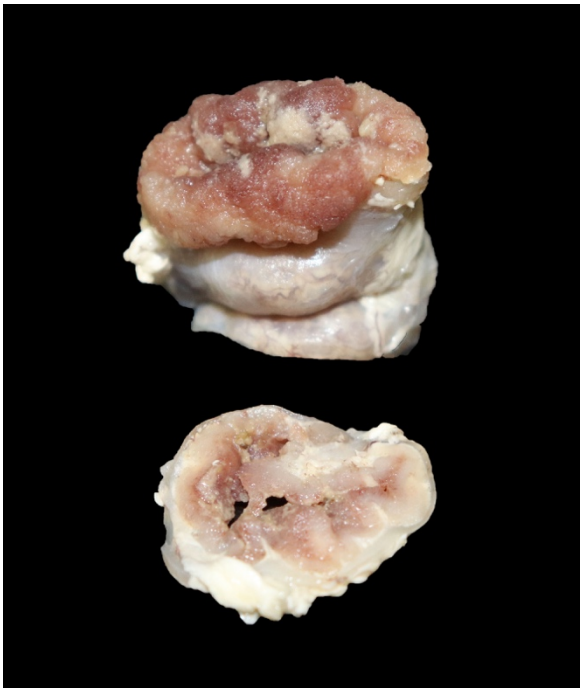
(PDCoV), porcine epidemic diarrhoea virus (PEDV), transmissible gastroenteritis virus (TGE), porcine respiratory coronaviruses, bovine viral diarrhoea virus (BVDV), classical swine fever (CSF) and African swine fever (ASF), and general mammalian virus isolation did not show any cytopathic effect. Blood was porcine circovirus 2 (PCV2) rt-PCR low positive at a level that was not consistent with porcine circovirus associated disease (PCVAD).

Microscopic description:
One unlabelled short segment of intestine was examined (pig 3). Differentiation between distal ileum, caecum and colon is difficult due to extensive pathology present.

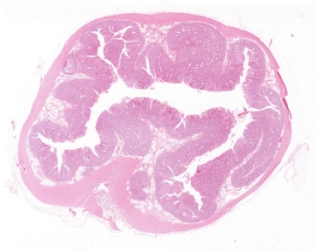
The mucosa is diffusely markedly thickened by pseudo-stratified, crowded and mitotically active crypt epithelium, with plump, vesiculated nuclei. Multifocally, villous tips are moderately to severely blunted and fused, or replaced by thick bands of cellular and karyorrhectic debris (necrosis), admixed with granular, lightly basophilic material (bacterial colonies), moderate infiltrations of lymphocytes, plasma cells, neutrophils and fewer eosinophils, and moderate amounts of hypereosinophilic, fibrillar material (fibrin). The mucosa is concurrently expanded by clear space (edema) and mild lymphoplasmacytic proprial infiltrates. Crypt lumens are often expanded by mucinous material and cellular debris (cryptitis). Foci of adenomatous epithelium sometimes herniate into depleted germinal centres of gut associated lymphoid follicles (Peyer's patches). Variably between sections there are numerous intraluminal, or occasionally proprially invasive, large (30-54 x 36-76 um), rounded, ciliated protozoa, with a paracentral macronucleus (consistent with *Balantidium coli* trophozoites).

Histochemical stains: Warthin-Starry Silver stain reveals large numbers of short, comma-shaped, argyrophilic bacteria, clustered apically within enterocytes, free within crypt lumens and occasionally within *B. coli* trophozoites (consistent with *L. intracellularis*).

Immunohistochemistry (IHC): *L. intracellularis*: There was strong intracellular immunoreactivity at the apical margin of epithelial cells, and to a



Cecum and colon, pig. On cross section, the marked mucosal proliferation markedly impinges on the lumen. (Photo courtesy of: State Veterinary Diagnostic Laboratory (SVDL), Elizabeth Macarthur Agricultural Institute, New South Wales, Australia).



Cecum and colon, pig. There is marked hyperplasia of the mucosa, which is thrown into rugose folds. (HE, 5X)

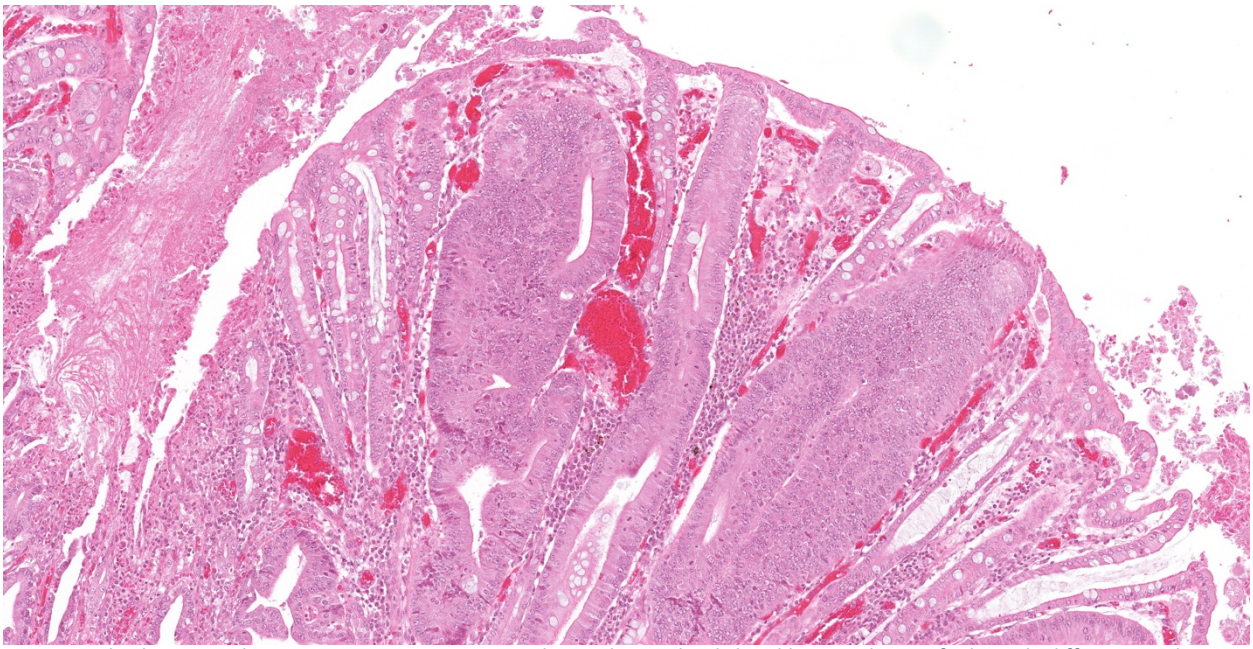
lesser extent within small numbers of macrophages in the lamina propria. *B. pilosicoli* and PCV2: There was no significant immunoreactivity within examined sections.

Contributor's morphologic diagnosis:
Intestine: enteritis, proliferative and fibrinonecrotizing, diffuse, severe, chronic, with lymphoid depletion and crypt herniation, cryptitis, intraepithelial, argyrophilic bacteria, aetiology consistent with *L. intracellularis*, and intralesional ciliated protozoal organisms, pig *(Sus scrofa domesticus)*

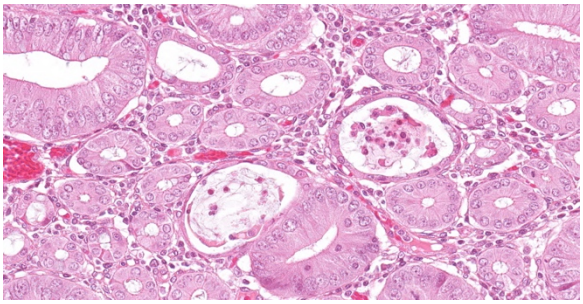
Contributor comments: Investigating the primary cause of diarrhoea and wasting in growing pigs is complicated by mixed infections, as in this case, where histopathology was used to diagnose porcine proliferative enteropathy (PPE), caused by LI, with background *B. pilosicoli* and PCV-2 infections.

LI is an obligate intracellular, microaerophilic, non-flagellated, non-spore forming, gram-negative, curved or S-shaped rod bacterium.[10,20-22] LI can infect a range of species including swine and foals, and less commonly donkeys, deer, sheep, rodents (e.g. guinea pigs, hamsters, mice, rats), rabbits, foxes, dogs, ferrets, emus, ostriches and non-human primates.[21,22]

The two main clinical manifestations of LI infection in pigs are porcine intestinal adenomatosis (PIA) and porcine haemorrhagic enteropathy (PHE). PIA is most common in post-weaning animals aged 2-5 months, and can progress to necrotic enteritis (NE), usually involving superimposed secondary bacterial infections, and then regional ileitis (RI) if the animal recovers from NE.[6,10,20-22] PHE is acute and most common in finishing pigs, young gilts and boars, typically 4-12 months of age, especially when naïve animals are introduced to a site of endemic LI infection.[6,22] Clinical signs of



Cecum and colon, pig. The mucosa contains numerous hyperplastic glands lined by pseudostratified poorly differentiated columnar epithelium. Glands contain several mitotic figures and no goblet cells. There is expansion of the lamina propria with lymphocytes, macrophages, and multifocal hemorrhage and a large area of necrosis (far left). (HE, 100X).



Cecum and colon, pig. There are crypt abscesses deep in the mucosa. (HE, 400X)

PIA vary from subclinical infection, to reduced growth rate with capricious appetite, to persistent diarrhoea, wasting and mortality,[18,20,21] with mortality rates usually being low and most often a consequence of secondary bacterial infections.[6] Main differential diagnoses include brachyspiral colitis, salmonellosis, colibacillosis, *Clostridium perfringens* type C, yersiniosis, coronaviruses and porcine circovirus-associated disease (PCVAD).[9,17,19,22] PHE is associated with acute to subacute intestinal haemorrhage, which may manifest as melena or haematochezia, weakness and pallor, or rapid death associated with exsanguination, with mortality rates reaching up to 50%.[6,20-22] The main differential diagnoses include swine dysentery (*B. hyodysenteriae*, *B. hampsonii*, and *B. suanatina*), salmonellosis, gastric ulcers and haemorrhagic bowel syndrome, which is often attributed to intestinal volvulus.[22]

Macroscopic lesions in PPE are most common in the terminal ileum and also occur in the cecum and colon.[20,21] Rarely infection may be restricted to the large intestine.[8] Gross lesions consist of ridges or plaque-like thickened areas that project above the normal mucosa and on the serosal surface the hyperplastic mucosa and edematous submucosa forms a reticular or cerebriform pattern of projections, which is virtually pathognomonic.[20-22] In NE there is more extensive mucosal necrosis, often with diphtheritic membranes and luminal fibrin casts.[9,20-22] PHE is characterised by overt or discrete areas of haemorrhage and ulceration, most common in the ileum.[20,21]

Following oral inoculation LI remains viable during passage through the stomach by utilizing systems that maintain pH homeostasis,[22] and within 12 hours can be demonstrated in mature villous tip enterocytes.[3,22] Within 5 days post inoculation the crypt epithelium becomes infested.[3,22] The reasons for its bias for the ileum are unknown, but it may relate to specific receptors, a favourable physiological environment, or mechanical reasons such as gut transit time.[22] Virulence factors associated with adhesion and entry have not been fully characterised, however the process is most likely dependent on host cell activity,[22] and probably involves a type 3 secretion system (T3SS), the genes for which are present in porcine LI isolates.[22]

There have been several proposed mechanisms of LI intracellular survival, including a *Salmonella* pathogenicity island 2 (SPI2)-related operon which may enable escape from endosome into cytosol, a sophisticated oxidative protection mechanism, and an ATP/ADP translocase used to exchange bacterial ADP for host cell ATP, thus exploiting the hosts energy pools.[15,22] Following vacuolar escape LI replicates in apical cytoplasm by binary fission, a process thought only to occur in actively dividing cells.[22] LI induces enterocyte proliferation,[22] although the exact host cell upstream cellular pathways leading to PE are unknown,[1,5] proposed mechanisms include the production of a cyclomodulin,[22] and simultaneous induction of Notch-1 signalling and attenuation of beta-catenin/Wnt pathways.[5] The latter may also be responsible for inhibiting the maturation of goblet cells,[5] which have also been shown to express lower levels of mucin 'MUC2' with LI infection, possibly altering the mucus barrier and helping to facilitate cellular invasion.[1] Eventually LI extrudes out of host cells or is released following apoptosis, then either infecting new enterocytes via the gut lumen, proprial ECM or possibly macrophages, or being shed in feces.[3,6,8,19,20,22]

The diarrhea observed in PPE is most likely due to loss of functional mucosal surface area, whereas wasting most likely relates to protein-losing enteropathy.[14,21,22] As the mucosal thickening in PIA becomes more extensive there is coagulative necrosis of the mucosa, allowing for colonization by pathogenic anaerobic large bowel flora and the formation of diphtheritic

membranes and fibrin casts.[21] It is unclear if development of PHE relates to the host, infectious dose, virulence of the infecting strain and/or other coinfections.[6] Experimental infection of gnotobiotic pigs has shown that PPE will not occur without undefined interactions with other gut bacteria, such as pathogenic *E. coli* and *Salmonella* sp.[21,22]

As in this case, multiple potential pathogens may occur in the same animals, and the relationships between PPE and other diseases are often complex, with LI causing alterations in the composition of the gut microbiome, and either directly or indirectly increasing the likelihood of coinfection with some pathogens such as *Salmonella enterica*, and decreasing the likelihood of others, such as PCV2.[6,12,17] Although *B. pilosicoli* was identified by PCR in this case, clinical signs and gross changes were not suggestive of porcine intestinal spirochetosis, which typically presents as a watery to mucoid diarrhea associated with typhlocolitis.[13,21] Histologic changes in this case were consist with LI, and not with *B. pilosicoli* which causes goblet cell hyperplasia, with organisms often forming a 'false brush border' of palisading upright bacteria perpendicular to enterocytes, or being observed within colonic crypts and goblet cells.[21] A flagellated protozoan, consistent with *Balantidium coli*, was also observed in this case. *B. coli* is a facultative pathogen of the intestinal lumen, usually associated with subclinical infection.[16] Following injury to the intestinal wall trophozoites may penetrate into the mucosa, usually in the cecum and colon, causing diarrhea, hematochezia and tenesmus.[16,21] In one study LI was identified in small numbers inside *B. coli* trophozoites in PPE cases.[7]

PIA does not usually feature a prominent inflammatory response, which may in part be due to LI downregulating CD2 expression, thereby minimising T cell activation.[21,22] In more chronically affected pigs, or in cases that progress to NE, more significant proprial mononuclear infiltrates may be observed.[22] Proprial histiocytosis, as well as depletion and necrosis of Peyer's patches and giant cell formation, may be observed both in PPE and PCV2-associated enteritis.[9] However the latter also differs from PPE histologically in many respects, including histiocytosis in lymphoid tissue, cytoplasmic inclusion bodies, and PCV2 IHC positive histiocytes in the lamina propria, submucosa, gut associated lymphoid tissue and within villous and crypt enterocytes.[9]

The gold standard for diagnosis of PPE is IHC using LI specific antibodies.[4,22] Other methods of characterizing spirochetal organisms on histologic section include in situ hybridization and electron microscopy.[6,20] Cultivation of LI is not routinely performed, and requires live tissues cultures.[20,21] Feces or tissue samples can be used for PCR and fecal smears can also be tested using immunoperoxidase staining.[4]

Contributing Institution:
State Veterinary Diagnostic Laboratory (SVDL)
Elizabeth Macarthur Agricultural Institute
New South Wales
Australia

JPC diagnosis:

1. Cecum: Typhlitis, proliferative, diffuse, severe, with multifocal ulceration, crypt herniation, and crypt abscessation, unknown breed, porcine.
2. Cecum, mucosa: Ciliates, few, etiology consistent with *Balantidium coli.*

JPC comment:
The contributor provides an excellent narrative for this disease and covers the topic comprehensively. This discussion was about manifestations of *L. intracellularis* in pigs, but it can affect other species as well.

L. intracellularis is the causative agent of equine proliferative enteropathy (EPE) of weanling foals in Scandinavia, which results in thickening of the small intestinal wall, and the ileum in particular. Clinical signs were similar to porcine patients, and included lethargy, anorexia, pyrexia, peripheral edema, colic, and diarrhea, with associated clinicopathologic changes of hypoalbuminemia and leukocytosis. This may represent a situation unique to Denmark due to the proximity of the discussed horses to high density pig farming operations, and no specific strain analysis has been performed to date.[2]

While exact pathogenesis has been elusive, the inflammatory response of infected cells has recently been characterized. By comparing control and infected animals, differentially expressed genes were isolated, and correlated well with the severity of lesions observed. Some important upregulated genes are *XDH* (encodes xanthine dehydrogenase), *MMP-7* (encodes matrix metalloproteinase-7), *TGM2* (encodes transglutaminase-2), and *OSM* (encodes oncostatin M). Xanthine dehydrogenase is an important regulator of inflammatory pathways, and ultimately results in the production of reactive oxygen and reactive nitrogen species. MMP-7 is important for activating a-defensin antimicrobial peptides and is often found to be overexpressed in colorectal cancers, causing an induced cellular proliferation. Oncostatin M is a cytokine associated with diseases with chronic inflammation, and transglutaminase-2 is also linked to cellular proliferation. The changes induced by *L. intracellularis* have similarities with some cancers, but once the infection is resolved, there is resolution and not neoplastic growth.[11]

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CASE 3: ND18-48 (4122555-00)

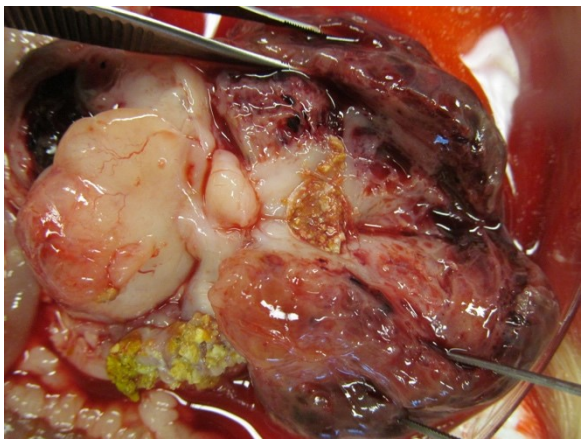
Signalment: 1-½ year old, Chat-Cre transgenic, female, Long Evans rat (*Rattus norvegicus*)

History: This was a rat used for training research staff on proper rat handling technique. The animal presented with ataxia, tachypnea, and a ruffled coat. There was no history of prior clinical abnormalities.

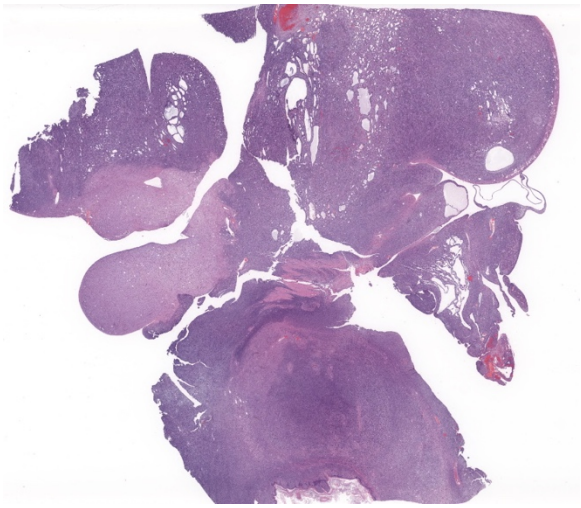
Gross Pathology:
On necropsy, the abdominal cavity was filled and expanded by 2 large coalescing adherent masses: The cranial mass was more solid, firm and tan measuring 4 cm x 2 cm x 2 cm while the second caudal mass measuring 4 cm x 5 cm x 2 cm was tan-white, moderate soft with multiple variable size cysts filled with brown fluid. The tan solid mass was found to be associated and firmly adherent to the lesser curvature of the stomach. There were small amounts of serosanguineous abdominal fluid. The masses compressed and displaced the diaphragm cranially. There was adhesion of the abdominal mass to the spleen.

Laboratory results:
Not pursued.

Microscopic description:
The submitted section consists of a large vaguely encapsulated, highly cellular and pleomorphic infiltrative neoplastic mesenteric mass that arises



Stomach and mesentery, rat. Two neoplastic masses are present within the abdomen. (Photo courtesy of: Massachusetts Institute of Technology, Cambridge, MA 02139)



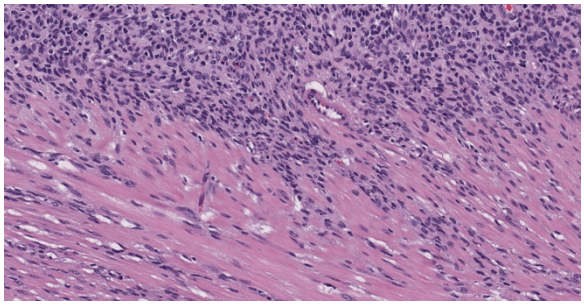
Stomach and mesentery, rat There are multiple sections of the neoplasm which are submitted for examination. (HE, 6X).

from the lamina propria of the squamous stomach with effacement of the submucosa and muscularis proper. The neoplasm has a pleomorphic appearance ranging from bland solid nodular more fibrous appearance to regions of high cellularity comprising of whorls or short intersecting fascicles of spindle to epithelioid cells in abundant hyalinized to vacuolated stroma. In general, the neoplastic cells don't have clearly discernible cell borders and contain variable amounts of eosinophilic to amphophilic cytoplasm and or fibrous stroma. The nuclei were small to moderately sized, oval or indented with a finely granular to vesiculated chromatin infrequently with a prominent nucleolus. In large sections of the mass, there are numerous variably sized pseudocystic or variably sized spaces with degenerate cells or basophilic material admixed with distended vascular channels filled with blood. Mitotic figures are 3-4 per 10 40X objective field.

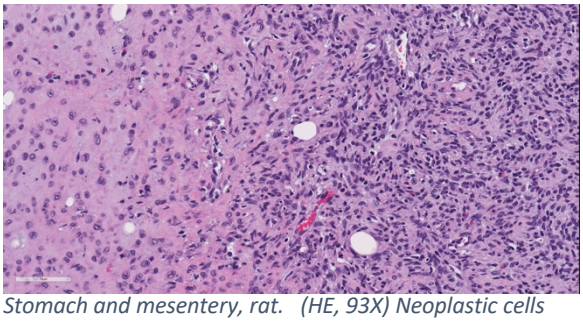
Immunohistochemistry:
The neoplasm was strongly positive for both c-KIT and Vimentin with sparse for S100 and was negative for pancytokeratin.

Contributor's morphologic diagnosis:
Stomach/Mesenteric masses**:** Gastrointestinal stromal tumor

Contributor's comment:
The most common mesenchymal tumors of the gastrointestinal tract in humans is gastrointestinal stromal tumors (GIST), however, spontaneous GISTs are rare in rats and primates.[2,3,9,11] In canines, these have been well characterized for their comparative morphology and biological behavior similar to humans.[4] Historically, they were classified as smooth muscle tumors such as leiomyoma and leiomyosarcoma, but it is now thought that these tumors arise from the cells of Cajal.[3,9] GISTs can occur anywhere along the gastrointestinal tract but are commonly found in the stomach. The appearance of these types of tumors can vary from soft, tan nodules to complex cystic masses depending on size and malignancy.[3,4] GISTs can be divided into 4 types including smooth muscle, neural, combined smooth muscle-neural and uncommitted.[3] For this case, due to the solid and cystic appearance and variable morphological pattern ranging from spindle to epithelioid, the differentials considered were GIST, adenocarcinoma, fibrosarcoma, leiomyosarcoma, mesothelioma, nerves sheath tumors and vascular origin tumors (lymphangiosarcoma, hemangiosarcoma). On the basis of its location (gastric location), histomorphologic features, and immunohistochemical expression pattern (diffuse strong c-KIT (CD117) and vimentin positivity, sparse S100 reactivity and lack of pancytokeratin expression), the abdominal masses were diagnosed as gastric origin- GIST. Smooth Muscle Actin (SMA), a marker of leiomyoma and leiomyosarcoma and cluster of differentiation (CD) 34 which can label both GISTs and smooth muscle origin tumors were not performed in this case. A strong c-KIT positivity is usually



Stomach, rat: Sections of stomach are largely effaced by neoplastic cells. Only small areas of mural smooth muscle remain. (HE, 259X).



Stomach and mesentery, rat. (HE, 93X) Neoplastic cells contain two distinct morphologies; at right, neoplastic cells are present in short streams and bundles reminiscent of neural tissue. At left, neoplastic cells are enmeshed in abundant eosinophilic stroma, have a moderate amount of basophilic homogenous cytoplasm and pleomorphic nuclei with prominent nuclei. (HE, 259X)

considered as the gold standard in the diagnosis of which is the gold standard marker for GISTs.[2,4,9] In this case, there was no distant metastasis other than mesenteric and splenic capsular involvement.

GISTs are positive for KIT tyrosine kinase receptor, which is also seen in the cells of Cajal, neural cells in the gastrointestinal tract that modulate gut motility. Around 70-80% of human GISTs are also positive for CD34, 30-40% of them are positive for α-smooth muscle actin, <5% are positive for desmin and S100-protein.[3,9] These tumors develop a gain of function mutation of the *KIT* gene commonly in exons 9, 11, 13, and 17. In humans, there are reports of familial GISTs due to mutations in the *KIT* and *PDGFRA* gene.[9] GISTs have been experimentally induced in rats using a duodenal reflux model but spontaneous findings in chronic studies using rats are usually under reported.[7,9] The prognosis of GISTs is based primarily upon the size and location of the tumor as well as its mitotic rate, distant metastasis and degree of necrosis.[4,9]

Contributing Institution:
Massachusetts Institute of Technology
Cambridge, MA 02139

JPC diagnosis:
Stomach, mesentery: Gastrointestinal stromal tumor, malignant, Long Evans rat, rodent.

JPC comment:
While this case was in a rat, there are documented cases of GIST in many species, including dogs, cats[10], horses, mice, and many others. Recent reports in horses have documented GISTs arising from the colon[6], as well as a discrete intra-abdominal mass with vascular connections to the transverse colon, mesocolon, jejunal mesentery, and the omentum, but still arising from the colon. That particular neoplasm was unusual in that it was characterized by immunoreactivity for desmin, as well as having multinucleated cells, and a predominately neuroid morphology of neoplastic cells.[8]

Because the majority of spindle cell neoplasms of the gastrointestinal tract in rats and mice used in research settings are diagnosed as smooth muscle tumors, and they cannot definitively be distinguished on H&E slides, immunohistochemistry is critical to determine the cell of origin. An examination of mouse and rat gastrointestinal spindle neoplasms in the National Toxicology Program found that there were few GISTs in rats, but that up to 60% of the previously classified smooth muscle tumors of mice demonstrated CKIT immunopositivity, and likely GISTs.[5]

While CD117 has been the gold standard to identify canine GIST, up to 5-10% of human GIST cases are negative for CD117. The immunohistochemical stain DOG1 (discovered on GIST protein 1) was shown to have a high sensitivity and specificity for human GISTs and was subsequently tested for canine GIST. Most canine GISTs had a similar IHC morphology for CD117 and DOG1, but some neoplasms were identified as GIST after negative CD117 and positive DOG1 results. Ultimately, DOG1 may have a higher sensitivity and specificity for canine GIST than CD117 but running both may provide the most information to the pathologist.[1]

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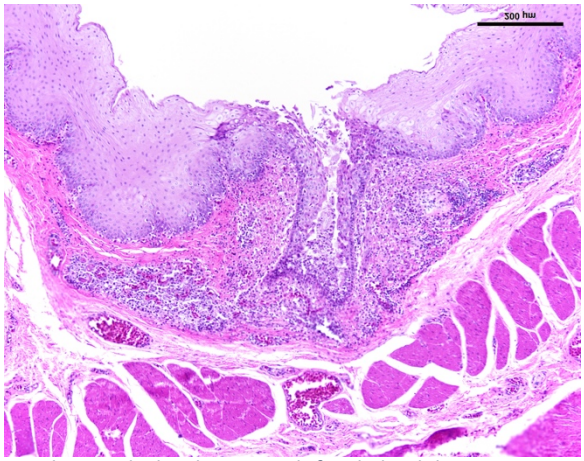
CASE 4: 17030960 (4101142-00)

Signalment: Two ducks: A 1-year-old, intact female, Muscovy duck, *Cairina moschata* and a 1-year-old, intact male, Muscovy duck, *Cairina moschata*

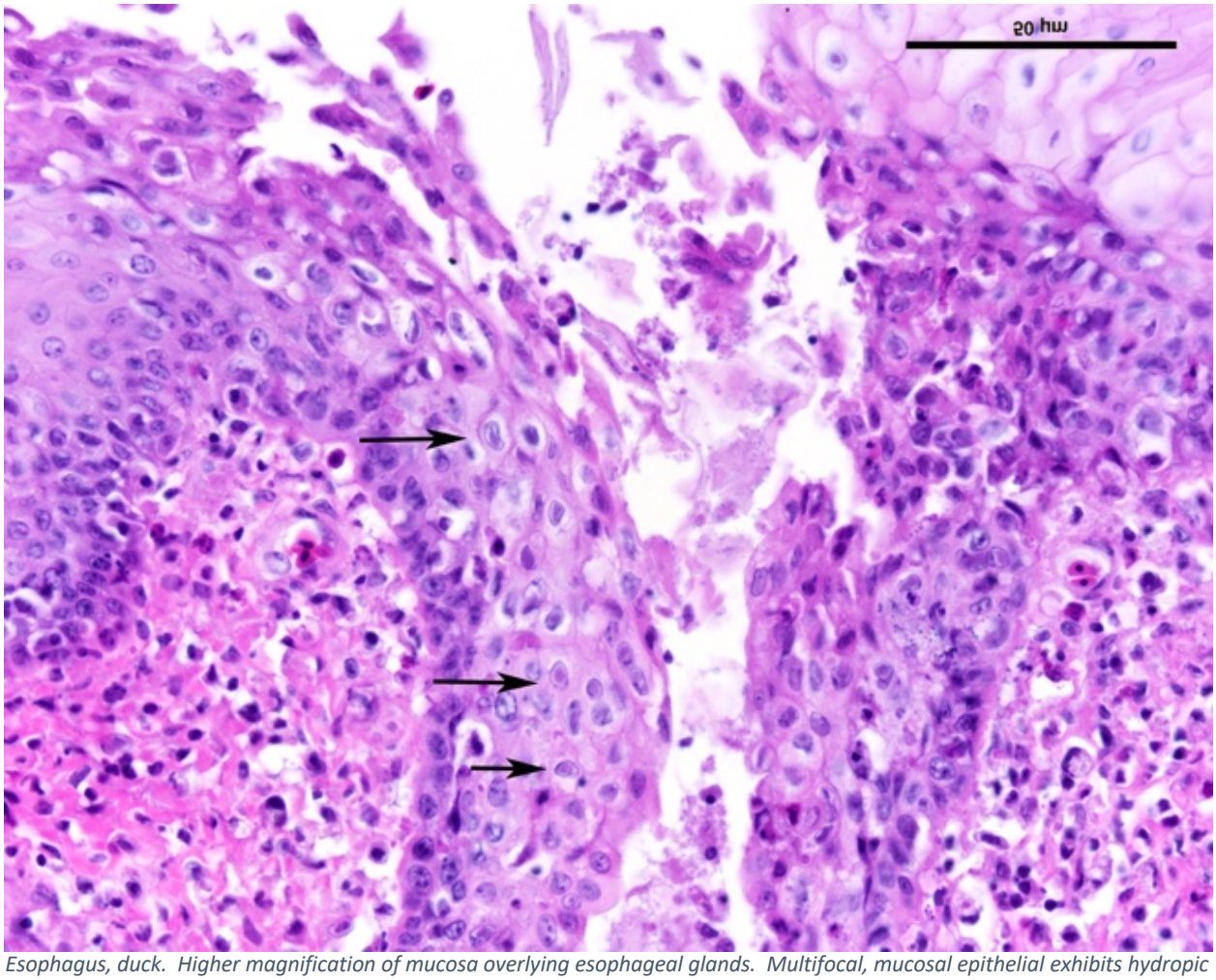
History:
Two ducks presented to the Oklahoma Animal Disease Diagnostic Lab on March 16th, 2017 for autopsy. Over a period of five days, approximately 25 ducks, all of which were Muscovy ducks, had died at Bethany Pond in the owner's front yard. She had cared for these animals since birth and had pet ducks that were not being affected; therefore, she raised concerns of intoxication rather than infectious agents. A similar die off had occurred previously in June of 2016 when approximately 24 ducks were found dead.

Gross Pathology:
Postmortem examination was performed on an adult female duck. The animal was in good body condition based on normal skeletal muscle mass and adequate adipose tissue stores. All tissues were mildly to moderately autolytic. No gross lesions were found.

Postmortem examination also found the adult male duck to be in good body condition with mild to moderate autolysis. The liver contained three,



Esophagus, duck. There is multifocal glandular necrosis and necrosis of the ostial mucosa. (Photo courtesy of: Oklahoma State University Center for Veterinary Health Sciences, Department of Pathobiology, http://cvhs.okstate.edu/Veterinary_Pathobiology). (HE, 100X)



Esophagus, duck. Higher magnification of mucosa overlying esophageal glands. Multifocal, mucosal epithelial exhibits hydropic generation, and intranuclear viral inclusions are present in several cells. ((Photo courtesy of: Oklahoma State University Center for Veterinary Health Sciences, Department of Pathobiology, http://cvhs.okstate.edu/Veterinary_Pathobiology). (HE, 200X)

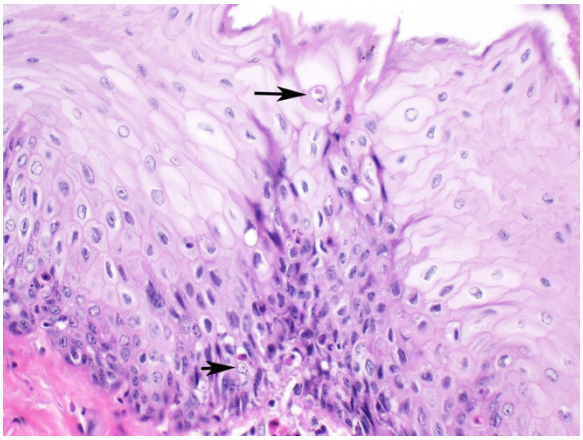
multifocal, discrete, 1mm diameter, firm, dark green foci on the capsular surface. No other gross lesions were observed.

Laboratory results:
All laboratory testing was completed at the National Wildlife Health Center in Madison, WI.

1. Results of RT-PCR for Duck Virus Enteritis (anatid herpesvirus – 1) performed on liver tissue were positive.
2. Virus isolation for Duck Virus Enteritis (anatid herpesvirus – 1) performed on liver specimens was negative.
3. Results of RT-PCR for Avian Influenza virus performed on trachea (routine screening on avian necropsy specimens) were negative.

Microscopic description:
Microscopic lesions are similar in both animals and are described together.

Esophagus: Multifocally, lumens of submucosal glands contain sloughed, necrotic epithelial cells admixed with apoptotic and necrotic macrophages and lymphocytes. Adjacent lymphoid aggregates also contain necrotic lymphocytes and cellular debris. Epithelium overlying the affected glands and lymphoid aggregates have ballooning degeneration in the stratum spinosum that progresses to necrosis with mucosal erosion or ulceration and accompanying inflammation. These areas of affected epithelium are typically continuous with the underlying gland. Large numbers of mucosal epithelial cells



Esophagus, duck. Higher magnification of mucosa overlying esophageal glands, demonstrative intranuclear viral inclusions (arrows). (HE,400X)

adjacent to these areas contain single 1 µm x 3 µm diameter, smudgy, magenta, intranuclear inclusion bodies that displace chromatin to the nuclear periphery, forming a halo.

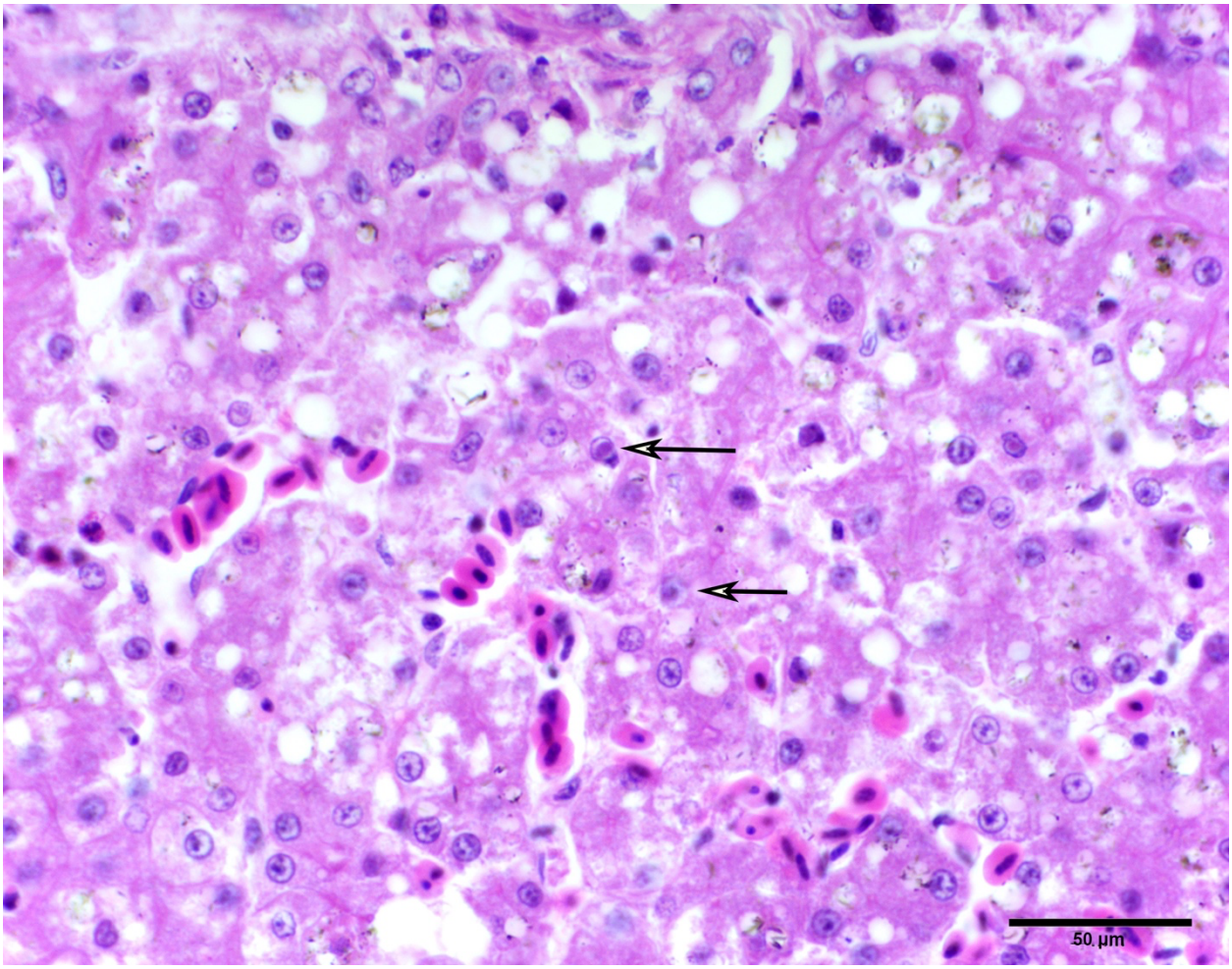
Liver: Randomly scattered to occasionally coalescing foci of hepatocellular necrosis are surrounded by hepatocytes that contain intranuclear inclusion bodies similar to those previously described. In areas not interrupted by hepatocellular necrosis, individual scattered hepatocytes have intranuclear inclusion bodies. Throughout the liver, hepatocytes have periportal to diffuse microvesicular and macrovesicular cytoplasmic vacuolation. Sinusoids are mildly ectatic and congested.

Proventriculus: Previously described intranuclear inclusions are within epithelial cells scattered throughout the section without additional lesions that are consistent on all slides.

Contributor's morphologic diagnosis:
Esophagus: Moderate to marked, acute, multifocal, erosive and ulcerative esophagitis with ballooning degeneration and submucosal glandular necrosis with intranuclear inclusion bodies consistent with anatid herpesvirus - 1

Liver: Moderate, acute, multifocal hepatocellular necrosis with intranuclear inclusion bodies consistent with anatid herpesvirus – 1 and periportal to diffuse hepatocellular vacuolation

Contributor's comment:
Duck Virus Enteritis (DVE), also known as Duck Plague is caused by anatid herpesvirus-1, a member of the alpha herpesviradae subfamily.[2] This infection affects a wide range of birds in the Anseriformes order with varying disease severity depending on viral pathogenicity, and age and species of the animals.[1,5] Mallard ducks along with Pintail ducks are considered to be the least susceptible to DVE and are implicated as carriers whereas Blue Winged Teal ducks and Muscovy ducks are highly susceptible to fatal infection.[1, 2,7] Young ducklings aged 2-7 weeks have been shown to be less severely affected in comparison to adult ducks.[7] DVE was first observed in 1923 and later described and distinguished from avian influenza in 1942.[1] Outbreaks have occurred worldwide, are seasonal, and tend occur near bodies of water. Clinical signs are variable and often nonspecific including sudden death in birds with good body condition, a drop in egg production, anorexia, depression, ruffled feathers, diarrhea, hematochezia, penile prolapse, nasal discharge, epiphora, photophobia, and periocular crusting.[1,5,6] Carrier birds can have sublingual ulcers.[6] Gross lesions typically include annular bands of necrotic intestinal mucosa with overlying bands of hemorrhage and pseudo membrane formation, hemorrhage into body cavities and/or into the intestinal lumen, and myocardial, hepatic, and ovarian hemorrhage with necrosis.[1,5,6] Microscopically, the gastrointestinal tract is primarily affected along with lymphoid organs; however, this virus is considered pantropic with lesions and intranuclear or intracytoplasmic viral inclusions found in multiple tissues.[2,5] DVE is transmitted horizontally from infected animals by either direct contact or indirectly through environmental contamination. Vertical transmission has only been observed experimentally.[1,2] Recovered birds are immune to reinfection; however, they become carriers with latent infections in the trigeminal ganglion and shed virus in times of stress, silently adding to environmental contamination.[5] Cell culture is the gold standard in diagnostics for DVE, although PCR and serology in combination with electron microscopy are also useful diagnostic tools.[5] There is no effective treatment for DVE and control relies heavily on management,



Liver, duck. Hepatocytes adjacent to areas of necrosis demonstrate intranuclear viral inclusions (arrows). (HE, 400X)

depopulation, and communication with state and federal agencies.[1] Live attenuated and killed vaccines are available for production animals but have not been distributed to the general public.

The outbreak within this flock spared Mallard ducks and killed all Muscovy ducks, which is consistent with the literature. Ducks in our case were also lacking gross lesions, which supports the sensitivity of this species to DVE. In 1987, Wobeser described a similar lack of gross lesions in Blue-Winged Teal ducks. Although the paper speculated that may have been due to a more pathogenic strain of virus, we hypothesize that the lack of gross lesions in these two species may be due to the more rapid disease course and death found in highly susceptible animals. This is also demonstrated in the multifocal distribution of the esophageal lesions in our ducks. The literature describes diffuse lesions in most species that have longer average times to death. Wobeser also documented microscopic lesions within the Blue-winged Teal ducks that mirror lesions found in our Muscovy ducks. This case is a nice representation of the disease process of DVE in highly susceptible species and demonstrates the pantropic nature of DVE.

Contributing Institution:
Oklahoma State University Center for Veterinary Health Sciences
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JPC diagnosis:

1. Esophagus: Esophagitis, necrotizing, multifocal, moderate with glandular epithelial necrosis, lymphocytolysis, and occasional intranuclear and intracytoplasmic viral inclusions.

2. Liver: Hepatitis, necrotizing, multifocal, mild to moderate, with intranuclear viral inclusions.
3. Liver, hepatocytes: Vacuolar degeneration, diffuse, moderate.
4. Proventriculus: Lymphocytolysis, diffuse, mild to moderate.

JPC comment:
During case discussion, some participants noted the presence of intracytoplasmic inclusions in addition to intranuclear inclusions. As the contributor correctly identifies, this herpesvirus may result in intranuclear and intracytoplasmic viral inclusions, which have been verified as viral nucleocapsids in cytoplasmic vacuoles.[3]

When this disease was first described in the Netherlands in 1923, it was thought that a new and distinct virus was causing disease in ducks, and the name "duck plague" was proposed, and adopted for many years. Subsequently, the disease is now identified as Duck Viral Enteritis (DVE), to separate the disease from fowl plague.[3]

Of the more than 300 viral encoded miRNAs that have been catalogued, more than 95% are encoded by viruses of the herpesvirus families. A comparison between vaccine and virulent Chinese strains of duck enteritis virus show the vaccine strain had 33 encoded miRNAs, while the virulent strain encoded 39 miRNAs. The virulent strain miRNAs formed a unique regulatory pathway as compared to the vaccine strain, indicating our understanding of the true pathogenesis is incomplete.[8]

Other hemorrhagic and necrotic lesions in anseriforms may be caused by fowl cholera (*Pasteurella multocida*), *Riemerella anatipestifer*, duck viral hepatitis (5 different viruses), coccidiosis, Newcastle disease, avian influenza, and goose herpesvirus. Differentials for intranuclear inclusions in duck hepatocytes would also include lead toxicity (common in waterfowl), and anatid adenovirus-2.

References:

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8. Wu X, Jia R, Zhou J, et al. Virulent duck enteritis virus infected DEV cells generate a unique pattern of viral microRNAs and a novel set of host microRNAs. *BMC Veterinary Research*. 2018;14:144.