Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2019-2020

C o n f e r e n c e 24

29 April 2020

CASE I: S62/16 (JPC 4084944). Tissue from a horse (*Equus caballus*).

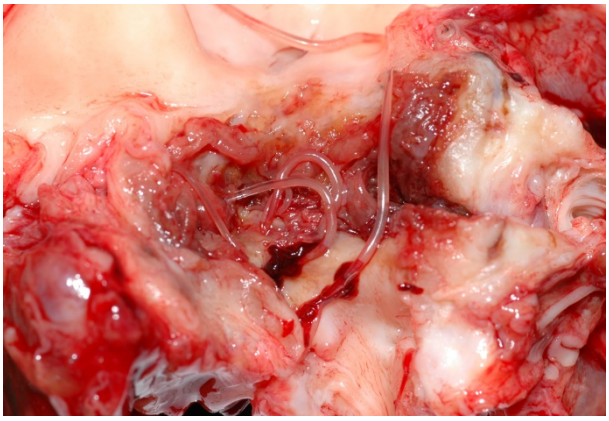
Signalment: Horse, pony, adult.

History: Incidental finding during scheduled euthanasia of several ponies in the course of a parasitological study.

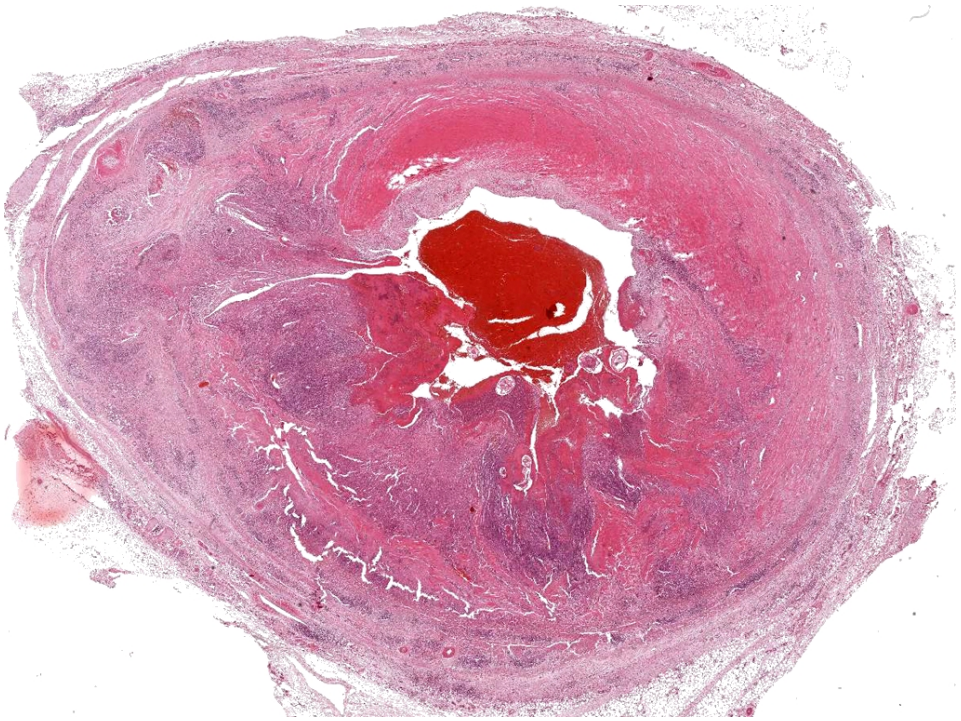
Gross Pathology: Mesenteric arteries of several ponies were thickened and stiffened. After opening of the vessels luminal larval nematodes were present in some animals. On cross sections the vessel lumina were narrowed and filled by thrombi with numerous entrapped grayish to white or transparent nematode larvae of approximately 1 to 2 cm in length and 1 mm in thickness.

Laboratory results: None.

Microscopic Description: Mesenteric artery: The endothelium, tunicae interna and media are widely replaced and expanded by abundant granulation tissue and massive inflammatory infiltrate. The infiltrate is composed of mainly macrophages, viable and degenerate neutrophils, eosinophils and few lymphocytes. Numerous thin-walled small caliber blood vessels, lined by plump endothelial cells and oriented perpendicular to the vascular lumen as well as increased numbers of mostly circular arranged fibroblasts and fibrocytes delineate the



Mesenteric artery, horse. A dilated aspect of one of the mesenteric arteries contains numerous large strongyle nematodes. (Photo courtesy of: Department of Veterinary Pathology, Freie Universitaet Berlin http://www.vetmed.fu-berlin.de/en/einrichtungen/institute/we12/index.html)



Mesenteric artery, horse. A dilated aspect of one of the mesenteric arteries contains numerous large strongyle nematodes. (Photo courtesy of: Department of Veterinary Pathology, Freie Universitaet Berlin http://www.vetmed.fu-berlin.de/en/einrichtungen/institute/we12/index.html)

inflammation in the periphery. The arterial lumen is partially occluded by an eosinophilic, amorphous material (fibrin thrombus) containing alternating layers of erythrocytes, viable and degenerate neutrophils, and fibrin (lines of Zahn) with multiple cross sections of nematode larvae. Larvae are up to 250 µm in diameter with a smooth cuticle, a pseudocoelom, platymyarian-meromyarian muscles, prominent lateral cords, and a large central intestine, lined by few multinucleated cells with a prominent brush border.

Contributor's Morphologic Diagnosis:

Mesenteric artery: Arteritis, severe, chronic-active, segmental, granulomatous and eosinophilic with (white) thrombus-formation and intralesional larval nematodes consistent with *Strongylus vulgaris*

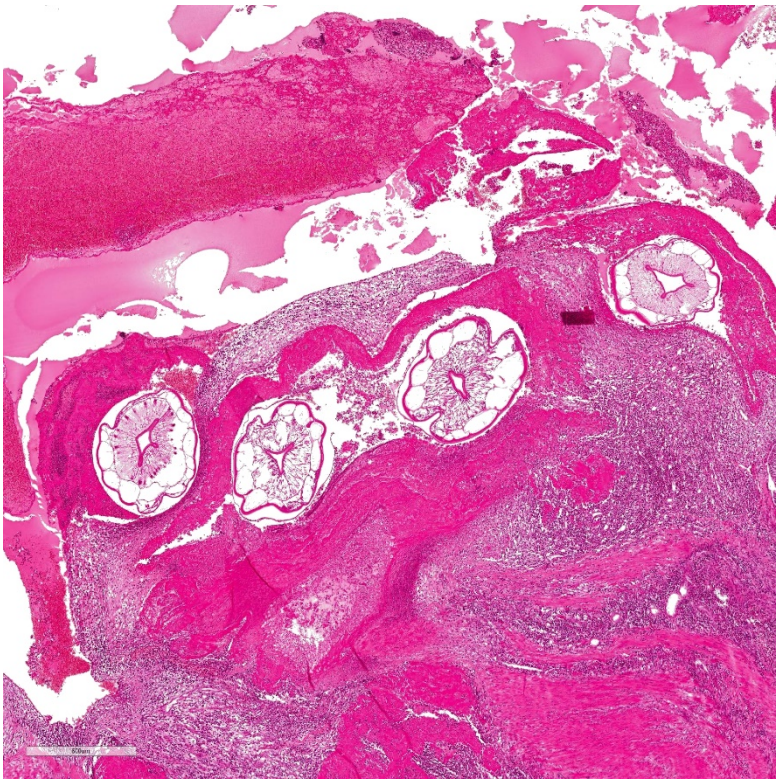
Contributor's Comment: Virtually all horses are affected by strongylid infections, of which the most damaging to the host is the infection with the large strongyle *Strongylus vulgaris*[4]. Infectious 3rd larvae of *S. vulgaris* are ingested and exsheath in the small intestine. After penetrating the intestinal mucosa, they molt to the 4th stage of 1 mm length and migrate in or along the intima to the cranial mesenteric artery. These larvae can cause large subserosal hemorrhages called *hemomelasma ilei*. After a maturation period of 3 to 4 months the 10 to 18 mm long 5th larvae (immature adult) returns to the wall of the cecum or colon via arterial lumen and encapsulate in the subserosa forming 5 to 8 mm large nodules. After rupture of the nodules with release into the intestinal lumen and another 1 to 2 months of maturation the adult nematodes produce eggs that are shed with the feces and develop outside the horse to the 3rd larvae.[6,7]

The two other common large strongyles affecting horses are *S. edentatus* and *S. equinus*. *S. edentatus* travels via the portal

system to the liver, molts to the 4th stage and re-enters the cecum via the hepatic ligaments. *S. equinus* molts in the walls of the ileum, caecum and colon, travels via the peritoneal cavity to the liver and later also the pancreas, molts to the 5th stage and return into the cecal lumen probably by direct penetration.[7]

Gross lesions can range from small visible tracts to thrombotic lesions as in this case. Thrombembolic intestinal infarction, especially of the large bowel and the colon can be the consequence.[7] Chronic infections may lead to thickening or even scarring of the affected vessels. Another possible consequence is weakening of the vessel walls with subsequent aneurysm-formation and rupture.[6] Debilitating disease with pyrexia, anorexia, depression, diarrhea and colic are more common in foals with high larval burden and consequence to toxic products from decaying larvae. A degree of host resistance can be slowly acquired under natural conditions, but all ages remain susceptible.[6] In addition to the aortic-iliac thrombosis *S. vulgaris* may also cause cerebrospinal nematodiasis.[7]

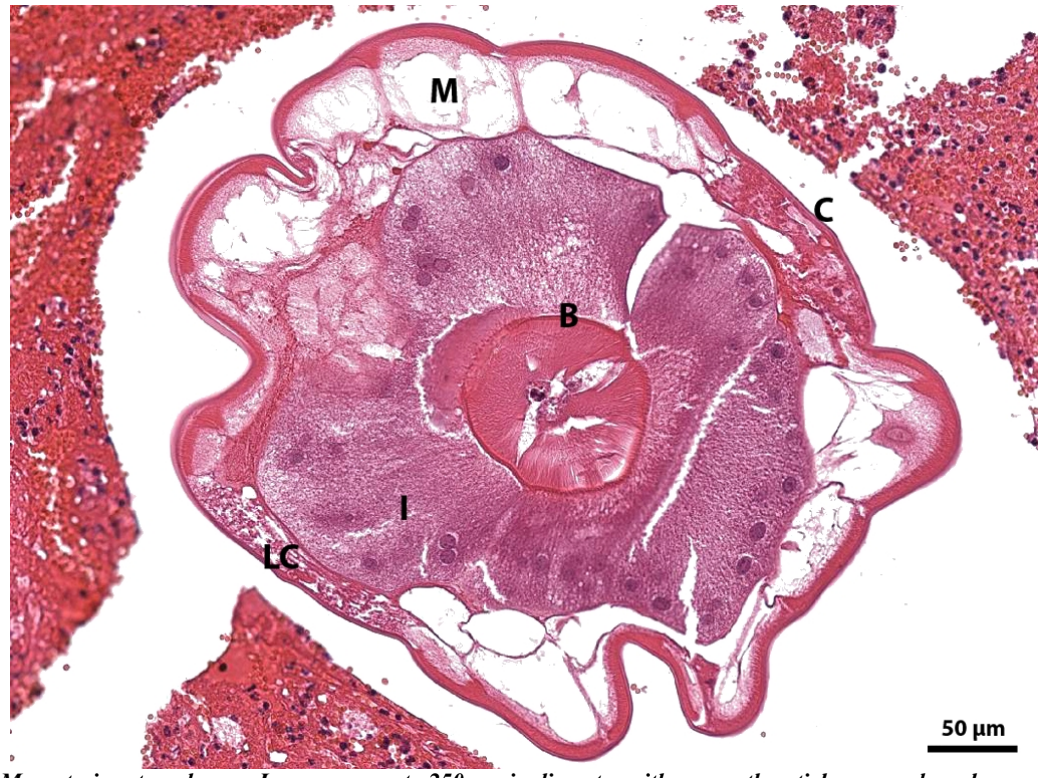
The number of infections decreased with improvement of anthelmintics but increase again due to upcoming resistances, especially in cases of infestation with small strongyles (cyathostominosis).[5] That's why the European Union made anthelmintics available on prescription-only basis[1]. This in turn led to an increased need of *intra vitam* diagnosis of nematosis. Especially the "traditional" egg count methods may be misleading as studies indicated that horses with counts below 100 eggs per gram can harbor cyathostomes burdens in the order of 100,000 luminal worms.[4] However. until now no reliable alternative method has been found.[1,5,6] That's why the number of strongylid infections seen in necropsy will possibly increase in near future.



Mesenteric artery, horse. Larvae are up to 250 µm in diameter with a smooth cuticle, a pseudocoelom, platymyarian-meromyarian muscles (M), prominent lateral cords (LC), and a large central intestine, lined by few multinucleated cells with a prominent brush border (B). (Photo courtesy of: Department of Veterinary Pathology, Freie Universitaet Berlin http://www.vetmed.fu-

Contributing Institution:

Department of Veterinary Pathology, Freie Universitaet Berlin



Mesenteric artery, horse. Larvae are up to 250 µm in diameter with a smooth cuticle, a pseudocoelom, platymyarian-meromyarian muscles (M), prominent lateral cords (LC), and a large central intestine, lined by few multinucleated cells with a prominent brush border (B). (Photo courtesy of: Department of Veterinary Pathology, Freie Universitaet Berlin http://www.vetmed.fu-berlin.de/en/einrichtungen/institute/we12/index.html)

http://www.vetmed.fu-erlin.de/en/einrichtungen/institute/we12/index.html

JPC Diagnosis: Mesenteric artery: Arteritis, proliferative, granulomatous and eosinophilic, transmural, chronic**,** diffuse, severe, with thrombosis and luminal larval strongyles.

JPC Comment: Even with the widespread use of ivermectin among horse owners, the classic lesion of *Strongylus vulgaris* have turned up in the WSC twice in the last 6 years (WSC 2013-2014 Conference 24 Case 4, and WSC 2017-2018 Conference 21 Case 4, 2017-2018.)

Before the widespread use of ivermectin, up to 90% of equine colic was attributed to strongyle infection (as 90% of horses with colic without obvious signs of obstruction had lesions associated with infection).

Arterial infarctions of the colon in animals with *S. vulgaris* infections have been well-documented in association with *S. vulgaris* infection and a source of puzzlement for veterinarians for many years. The lesion occurs in a small number of animals with remodeled mesenteric arteries, while the vast majority of infected do not show evidence of infarction. Several theories have been put forth about their cause, but none proven. It is compelling to think that pieces of the large thrombus in mesenteric arteries might break off and embolize to the affected regions, but its occurrence has never been documented. Another theory is vasospasm of the colonic arteries due to vasoactive substances liberated by the larvae or components of the thrombus (similar to the enhancement of ischemia induced in human myocardial infarcts by the thromboxane liberated by

platelets). Yet another theory is that the thickened arterial walls place pressure on the autonomic plexi, interfering with gut innervation.[7]

As mentioned by the contributor, reliable antemortem diagnosis of *S. vulgaris* infection is a continuing problem. Within the last few years, several potential antemortem diagnostic techniques have been proposed. Real-time identification of *S. vulgaris* antigens in fecal samples have been shown to be more effective in identifying infected animals than larval isolation techniques.[3] Recently an ELISA test measuring antibodies to recombinant *S. vulgaris* SXP protein was shown to be 73.3% sensitive and 81.1% specific for *S. vulgaris* infection.[2]

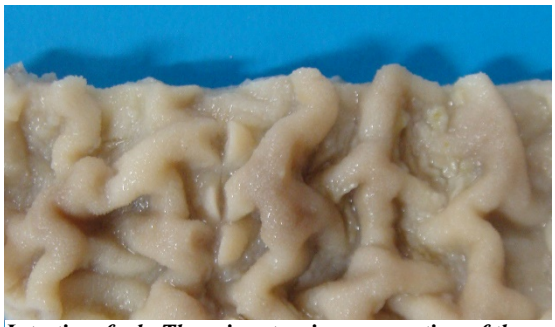
References:

1. Andersen UV, Howe DK, Olsen SN, Nielsen MK: Recent advances in diagnosing pathogenic equine gastrointestinal helminths: the challenge of prepatent detection. *Vet Parasitol* 2013; 192: 1-9.
2. Anderson UV, Howe DK, Dangoudoubiyam S, Toft N, Reinemeyer CR, Lyons ET, Olsen SN, Monrad J, Nejsum P, Nielsen MK. SvSXP: a Strongylus vulgaris antigen with potential for prepatent diagnosis. *Parasit Vect* 2013; 6:84.
3. Kaspra A, Pfister K, Nielsen MK, Silaghi C, Fink H, Scheuerle MC. Detection of Strongylus vulgaris in quine faecal samples by real-time PCR and larval culture - method comparison and occurrence assessment. *BMC Vet Res* 2017; 13:19, doi: 10.1186/s12917-016-0918-y.
4. Nielsen MK, Baptiste KE, Tolliver SC, Collins SS, Lyons ET: Analysis of multiyear studies in horses in Kentucky to ascertain whether counts of eggs and larvae per gram of feces are reliable indicators of numbers of strongyles and ascarids present. *Vet Parasitol* 2010; 174: 77-84.
5. Nielsen MK, Pfister K, von Samson-Himmelstjerna G: Selective therapy in equine parasite control--application and limitations. *Vet Parasito* 2014; *l* 202: 95-103.
6. Robinson WF, Robinson NA: Cardiovascular System. In: Jubb, Kennedy and Palmer's Pathology of Domestic Animals: Sixth Edition, pp. 1-101.e101. 2015
7. Uzal FA, Plattner BL, Hostetter JM: Alimentary System. In: Jubb, Kennedy and Palmer's Pathology of Domestic Animals: Sixth Edition, pp. 1-257.e252. 2015.
8. White NA: Thromboembolic colic in horses. *Comp Contin Educ* 1985; 7: S156-S163.

CASE II: Case 2 (JPC 4048931). Tissue from a horse (*Equus caballus*).

Signalment: 7 month-old, male, thoroughbred, horse (*Equus caballus*)

History: Foal tissue samples were submitted from a farm with a herd of about 300 horses of different ages and sexes. Foals were weaned between four to seven months of age. After weaning, all foals were grouped in batches of about 35 animals based on age. The facilities where the foals were daily handled were the same used for the management of other animals of different ages.



Intestine, foal. There is extensive corrugation of the intestinal mucosa. (Photo courtesy of the Veterinary School. Universidade Federal de Minas Gerais. (www.vet.ufmg.br)

Every year cases of diarrhea were reported with variable severity and characteristics in foals of different ages. Thirty-nine foals, nine from generation 2011 and 30 from generation 2012, showed clinical signs of pasty diarrhea evolving to liquid diarrhea. The body temperature varied between 39.5 to 41.0°C within 48 hours, in addition to lack of appetite and dehydration. In foals with three to six months of age, hypoproteinemia associated with submandibular edema were frequently observed. The duration of the clinical signs was from a few days to a few weeks.

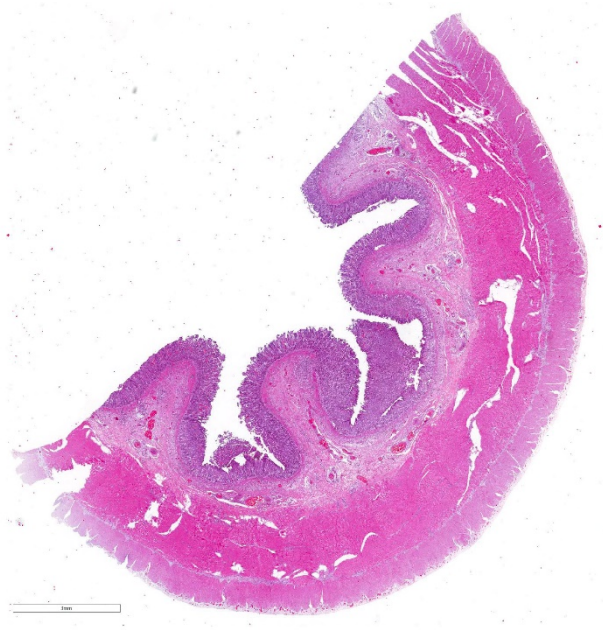
Seven of 39 foals had clinical signs of diarrhea and three of these animals died. A seven-month-old foal died four days after the onset of clinical signs and intestinal samples were submitted to the Laboratory.

Gross Pathology: Grossly, large amounts of blood-tinged fluid was observed in the peritoneal cavity. The serosa of the small intestine was hyperemic. Thickening of the intestinal wall was associated with a clear corrugation and thickening of the mucosa folds with consequent reduction of intestinal lumen.

Laboratory results: Eleven fecal and serum samples from this group of foals were also submitted to the laboratory. All samples were negative for the detection of *Cryptosporidium*, *Salmonella* spp. and, *Clostridium perfringens*. Three foals were seropositive for *Lawsonia intracellularis* (1:60 – Immunoperoxidase monolayer assay). All foals were negative for *L. intracellularis* by PCR in fecal samples.

Immunohistochemistry staining using *L. intracellularis* specific antibodies demonstrated antigen labeling at the cytoplasmic apex of enterocytes and inside macrophages in the lamina propria of the duodenum, ileum and large intestine.

Microscopic Description: Histological analysis of the duodenum, jejunum, ileum and large intestine demonstrated enterocyte hyperplasia of the crypts associated with an



Intestine, foal. A section of small intestine is presented for examination. At subgross magnification, there is marked villar blunting, and expansion of the submucosal by marked edema. (HE, 6X)

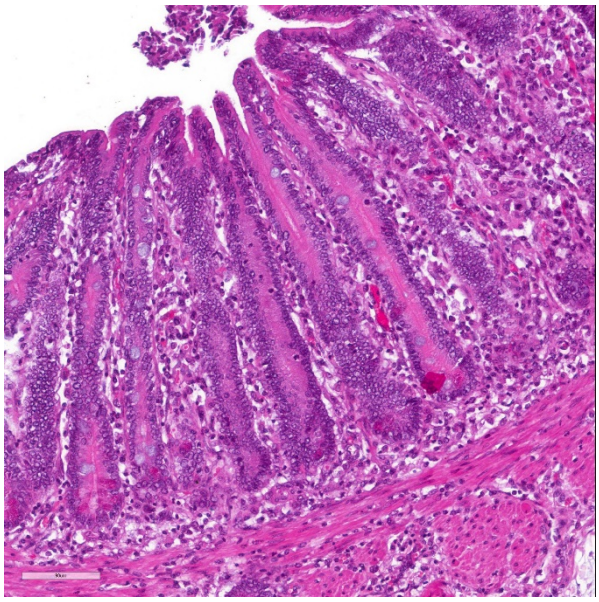
intense diffuse marked decrease in the number of goblet cells. Rare crypts were dilated and the lumen filled with cell debris and neutrophils (crypt abscess). In addition, crypts were present in some areas of the submucosa.

Contributor's Morphologic Diagnosis:

Small intestine, horse: Moderate diffuse proliferative enteropathy with mild multifocal adenomatosis.

Contributor's Comment: Reported clinical signs, gross and microscopic findings, all consistent with the literature[11,13] and associated with IHC positive signal for *L. intracellularis,* allowed us to reach the diagnosis of equine proliferative enteropathy (EPE). Unlike pigs, in which lesions and immunostaining are more concentrated in the final third of the small intestine, in horses they can also be found in the duodenum,[9] as reported in this case. The lesions of the large intestine are less frequent, but in our case, histologic lesions compatible with EPE were also found in colon.

Affected animals had ages ranging from a few days to 21 months of age; however, diarrhea associated with submandibular edema (consequent of the hypoproteinemia) was observed more frequently in foals from three to six months old, the age group in which these animals are more susceptible and affected by *L. intracellularis*.[8,9] This predisposition is probably associated with the decline of maternal antibodies,[7] as well as stressors such as weaning, the move to new paddocks and barns, worming and/or vaccination programs and/or early

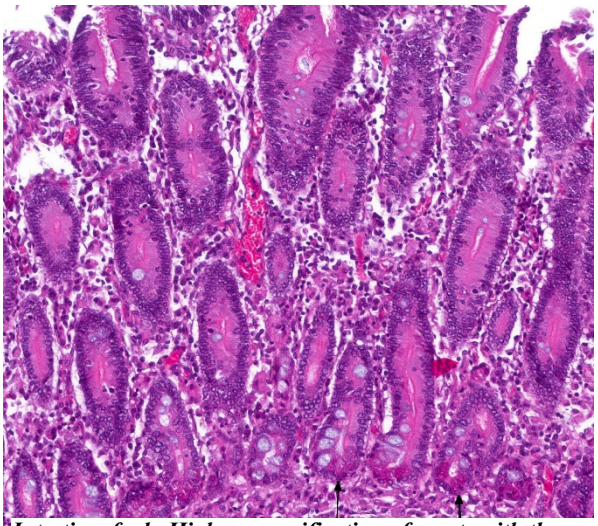


Intestine, foal. There is marked blunting of villi, resulting in a crypt:villus ratio of 1:5 or higher. Crypts are elongated with numerous mitotic figures. An infiltrate of macrophages and fewer lymphcoytes, eosinophils, and neutrophils separates crypts and lifts them off of the underlying muscularis mucosa. (HE,150X)

conditioning programs,[4] which were reported in this farm.

Animals with hypoproteinemia were treated with erythromycin, with clinical improvement in some of these animals. Treatment with erythromycin was effective for EPE in other reports[2,8] and is considered one of the treatments of choice for suspected cases of the disease. Disease progression varies from days to weeks, and the prognosis is good, if timely diagnosis and appropriate treatment are performed. Late diagnosed or untreated cases lead to death.[10]

Seropositivity for *L. intracellularis* in animals negative by PCR in fecal samples can be explained by the sensitivity of the last technique and/or by the course of the infection. There are many PCR inhibitors in fecal material that reduces the sensitivity of



Intestine, foal. Higher magnification of crypts with the expansion of the lamina propria by inflammatory cells. In areas of ulceration, higher numbers of neutrophils are present. Paneth cells (only seen in the equine small intestine) are present at the bottom of crypts and contain numerous brightly eosinophilic granules (black arrows). (HE, 242X)

the technique; as a result, the amount of shed bacteria in the feces could be below the detection threshold. In addition, serum antibodies against *L. intracellularis* last much longer then the bacteria fecal shedding.[10]

Contamination of colts in this report may have occurred through the feces of other positive horses due to daily use of the same facilities for all ages. *L. intracellularis* subclinical infection seems to be common in horses and fecal shedding of viable bacteria can be a source of infection for susceptible foals.[5,11] Wild and domestic animals, like dogs, cats, opossums (*Didelphis* spp.), and bush dogs (*Canis thous*) sighted on the property, can also be sources of contamination. Bacterial DNA has been detected in the feces of domestic and wild animals trapped in farms with the occurrence of EPE.[11]

The EPE is present in horse farms in Brazil and despite the report of a clinical case of the disease[6] and the detection of *L. intracellularis* in feces and serology in foals in other Brazilian states,[5] EPE remains neglected in the differential diagnosis with other enteric disease in foals.

Contributing Institution:

Veterinary School. Universidade Federal de Minas Gerais. (www.vet.ufmg.br)

JPC Diagnosis: 1. Small intestine: Enteritis, proliferative, diffuse, marked with villar blunting, crypt herniation, histiocytic and eosinophilic enteritis and submucosal edema.

2. Mesentery: Fat atrophy, diffuse, moderate with lymphohistiocytic steatitis.

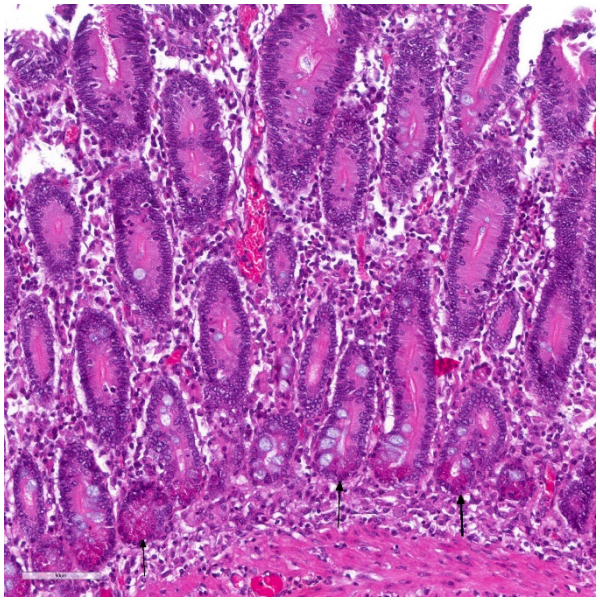
JPC Comment: *Lawsonia intracellularis* is a gram-negative, non-spore-forming, obligate intracellular bacterium named after Dr. Gordon Lawson, a long-term researcher at the University of Edinburgh on porcine proliferative enteropathy and the first to grow the bacterium in pure culture in 1993. This curve-shaped bacterium lives within the apical cytoplasm of a number of mammalian species, as well as chickens and ostriches, but has not been identified as an infectious agent in humans. A list of species which it has been identified in includes ferrets, swine, horses, dogs, rats, sheep, deer, ratites, nonhuman primates, and guinea pigs.[14] There are a number of subtleties of infection between species, with ferrets affected in the colon rather than small intestine, and with species like the rabbit and horse having a more profound inflammatory component. Marked

glandular proliferation of epithelium (historically referred to as "adenomatosis") characterizes the infection in hamsters and some ages of swine. Cross-species infections are often unpredictable – for example, hamsters may be infected with swine isolates (but not horse isolates) while rabbits may be infected with horse-derived strains (but not those of swine).[12,14]

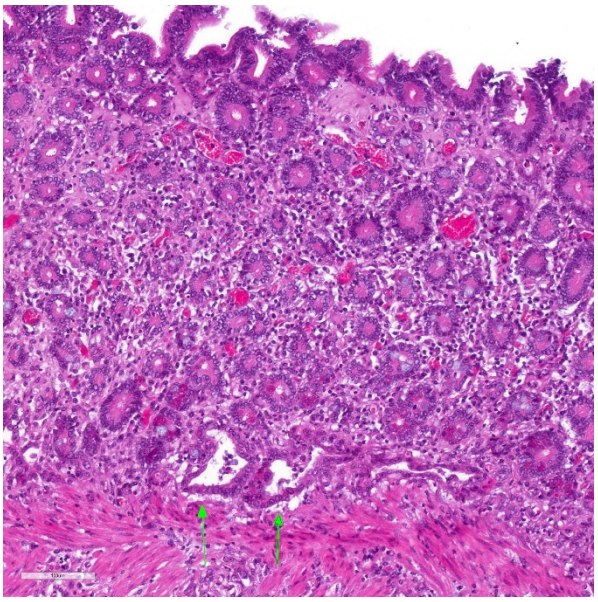
The bacterium possesses a single flagellum which provides an important boost to pathogenicity by allowing it to penetrate the mucus layer in the early stages of infection. Internalization of the bacilli within apical enterocytes occurs within 3 hours, but as of yet, the events associated with this important step have not been characterized.[14] The bacilli induces cellular proliferation of crypt epithelium, and lives free within the cytoplasm, undergoing binary fission and replication itself within 2-6 days. Infected cells may release bacilli into the crypt lumen via balloon-like cytoplasmic protrusions, and these bacilli can then infect adjacent enterocytes.[14]

In horses, the disease is referred to as equine proliferative enteropathy (EPE). Primarily affecting foals around weaning (2-8 months), it may also be seen in adult animals. Weight loss, hypoalbuminemia, peripheral edema, colic, diarrhea and leukocytosis are seen (but may also be seen in other gastrointestinal diseases of young horses such as infection with *Parascaris equorum* and cyathostomiasis), and ultrasound reveals prominently thickened loops of small intestine (resulting from the mucosal thickening and submucosal edema[3] evident on this slide).

Real time PCR is used to identify the bacterium in feces and is a very useful antemortem diagnostic test. Affected horses shed bacteria for 14-21 days following active infection, as compared to pigs, which shed bacilli up to 12 weeks. (This is why subclinical infections in the pig are much more readily identified than in horses.)[14] Identification of bacilli within crypt enterocytes may be done at autopsy, but some cases may yield negative results in PCR-positive animals.[3]



Intestine, foal. Multifocally, hyperplastic crypts herniate into the underlying mucosa. (HE, 50X)



Intestine, foal. Markedly inflamed segment of intestine with crypt abscesses (green arrows). (HE, 144X)

While PPE is considered a proliferative and inflammatory intestinal disease resulting in ill thrift and hypoproteinemia, a 2013 report by Arroyo et al.[1] described an outbreak of ulcerative and necrohemorrhagic disease in weanling foals more reminiscent of the hemorrhagic enteropathy seen as a variant of *L. intracellularis* infection seen in swine (known as porcine hemorrhagic enteropathy.) These foals, in addition to ulceration, hemorrhage, and the presence of the organism in 3 of 5 cases) failed to demonstrate the marked crypt hyperplasia which is common in horses and most other species. The two other foals in this group were negative for organisms on WS and immunohistochemistry, but were PCR-positive on antemortem fecal samples.[1]

Another mystery is how foals are infected. Exposure to swine is uncommon in horse farms today. One recent study examined rodents and stray cats in the vicinity of horse farms and found a 28% per cent detection rate for cats (via serology and PCR) and variable but lesser rates for a range of rodents. The range of infected animals varied greatly between farms, but suggests wild rodents or other mammals as potential sources on infection.[7]

References:

1. Arroyo LG, ter Woort F, Baird JD, Tatiersky L, DeLay J, van Drewuel T. *Lawsonia intracellularis*-associated ulcerative and necrohemorrhagic enteritis in 5 weanline foals. *Can Vet J* 2013; 54:853-858.
2. Bihr TP. Protein-losing enteropathy caused by *Lawsonia intracellularis* in a weanling foal. *Can Vet J.* 2003;44:65-66.
3. Bohin AM, Olsen SN, Laursen SH, OHman A, van Galen G. *Lawsonia intracellularis*-associated equine proliferative enteropathy in Danish weanlig foals. *Acta Vet Scand* 2019; 61:12 doi 10.1186/s13028-019-0447-3.
4. Frazer ML. *Lawsonia intracellularis* infection in horses: 2005–2007. *J Vet Intern Med.* 2008;22:1243–1248.
5. Guimarães-Ladeira CV, Palhares MS, Oliveira JS, et al. Shedding and serological cross-sectional study of *Lawsonia intracellularis* in horses in the state of Minas Gerais, Brazil. *Equine Vet. J.* 2009;41(6):593-596.
6. Guttmann PM, Viscardi V, Lessa DAB, et al. Equine Proliferative Enteropathy Caused by *Lawsonia intracellularis* in a Foal in Brazil. *J. Equine Vet. Science.* 2014;34:701-703
7. Hwang JM, Seo MJ, Yeh JY. *Lawsonia intracellularis* in the feces of wild rodents and stray cats captured around equine farms. *BMC*

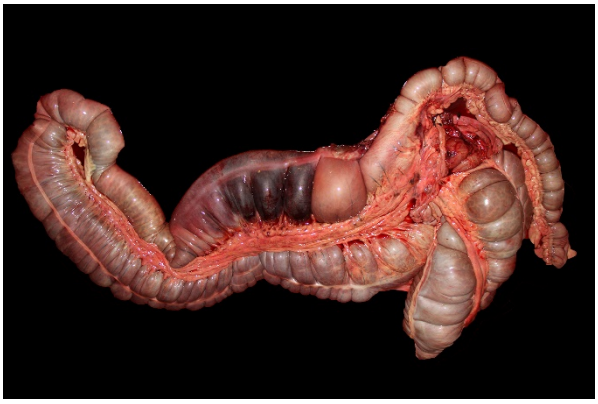
Vet Res, 2017; 13:233 doi 10.1186/s/s12917-017-1155-8
8. Lavoie JP, Drolet R, Parsons D, et al. Equine proliferative enteropathy: a cause of weight loss, colic, diarrhea and hypoproteinaemia in foals on the breeding farms in Canada. *Equine Vet J.* 2000;32(5):418-25.
9. Pusterla N, Gebhart C. *Lawsonia intracellularis* infection and proliferative enteropathy in foals. *Vet Microbiol.* 2013;167(1-2):34-41.
10. Pusterla N, Higgins JC, Smith P, et al. Epidemiological survey on farms with documented occurrence of equine proliferative enteropathy due to *Lawsonia intracellularis*. *Vet Rec.* 2008;163(5):156-158.
11. Pusterla N, Wattanaphansak S, Mapes S, et al. Oral Infection of Weanling Foals with an Equine Isolate of *Lawsonia intracellularis*, Agent of Equine Proliferative Enteropathy. *J Vet Intern Med* 2010;24:622–627.
12. Sampieri F, Vannucci FA, Allen AL, Pusterla N, Antonopoulos AJ, Ball KR, Thompson J, Dowling PM, Hamilton DL, Gebhart CJ. Species specificity of equine and porcine *Lawsonia intracellularis* isolates in laboratory animals. *Can J Vet Res* 2013: 77:261-272.
13. Vannucci FA, Pusterla N, Mapes SM, et al. Evidence of host adaptation in *Lawsonia intracellularis* infections. *Vet Res*. 2012;20:43-53.
14. Vannucci FA and Gebhart CJ. Recent advances in understanding the pathogenesis of Liawonia intracelullaris infections. *Vet Pathol* 2014; 51(2) 465-477.

CASE III: S1706449 (JPC 4117527)**.** Tissue from a horse (*Equus caballus*).

Signalment: Two-year-old Quarter horse gelding

History: Colic of unknown duration which could not be controlled with non-steroidal anti-inflammatory drugs (banamine and others) and was euthanized.

Gross Pathology: The carcass was in good nutritional condition, well-muscled and with adequate subcutaneous, perirenal and pericardial adipose tissue. The abdomen contained approximately 1 liter of red, thin, opaque fluid. Affecting three quarters of the right dorsal colon, extending distally from the diaphragmatic flexure, were the following changes: the serosa was diffusely red to dark red; the wall was transmurally thickened up to 2 cm and dark red and wet; the mucosa was mottled red to dark red to brown with a diffusely corrugated appearance; and the ingesta was semi-liquid green plant material that was red-tinged. There was a sharp line of demarcation between this region and the distal one quarter of the right dorsal colon, which had a slightly less thickened wall and diffusely tan serosa and mucosa). There was extensive petechiation of the epicardial surfaces of the atria and ventricles of the heart. No other significant gross abnormalities were observed in the rest of the carcass.



Right dorsal colon, horse. Segmentally, three-quarters of the right dorsal colon is distended and edematous with marked reddening of the serosa and a sharp line of demarcation between the affected area and the unaffected quarter. (Photo courtesy of: California Animal Health and Food safety Laboratory system, San Bernardino branch, University of California, Davis.)

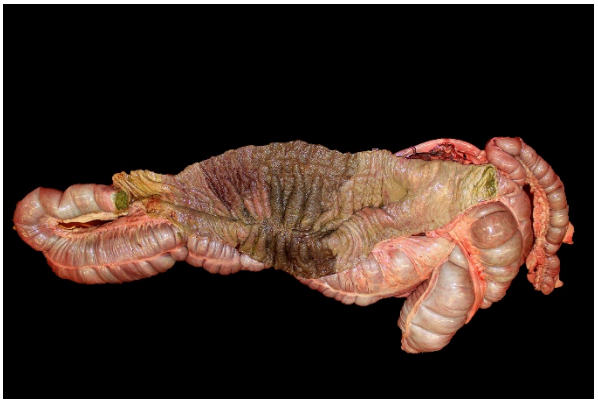
Laboratory results: Aerobic and anaerobic culture of colon tissue and content grew large mixed flora; culture of colon content for *Clostridium difficile* was negative. ELISAs for toxins A and B of *C. difficile* and for alpha, beta and epsilon toxins of *Clostridium perfringens*, respectively were negative. Salmonella culture and PCR were negative on colon tissue. Aerobic culture of liver and lung grew small numbers *of Streptococcus equi* ssp. *zooepidemicus* and mixed flora.

Microscopic Description: Colon: Diffusely, there is transmural congestion, edema and hemorrhage. The whole thickness of the mucosa is necrotic and presents lymphoplasmacytic and neutrophilic infiltration. The mucosal and submucosal vasculature presents fibrin thrombi and thrombotic vessels show fibrinoid necrosis and infiltration with viable and degenerate neutrophils. These vessels are frequently surrounded by hemorrhage and viable and degenerate leukocytes. The sub-mucosa is greatly expanded by homogeneous eosinophilic edema and diffuse infiltration of lymphocytes, plasma cells, macrophages and neutrophils. The lymphatic vessels are dilated and contain large amounts of fibrin, red blood cells and neutrophils, both viable and degenerate. Large numbers of mix bacteria admixed with fibrin and cell debris cover the denudated superficial mucosa. The serosal blood vessels are also thrombotic and present fibrinoid necrosis.

Contributor's Morphologic Diagnosis:

Colon, right dorsal: Colitis, severe, diffuse, acute, necrotizing with severe fibrinonecrotizing vasculitis, fibrin thrombosis and massive submucosal edema and congestion

Contributor's Comment: Microscopic examination confirmed severe necrotizing right dorsal colitis with extensive vasculitis and many fibrin thrombi. Given the morphologic diagnosis, location, exclusion of *C. difficile*, *C. perfringens* and *Salmonella spp*., and history of banamine administration, this is likely consequence of

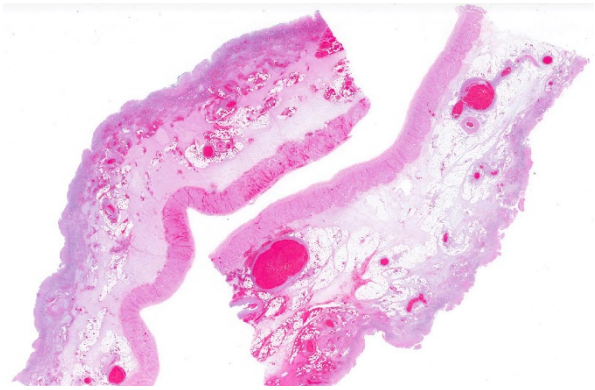


Right dorsal colon, horse. The mucosa of the affected segment is corrugated and mottled dark red to brown. (Photo courtesy of: California Animal Health and Food safety Laboratory system, San Bernardino branch, University of California, Davis.)

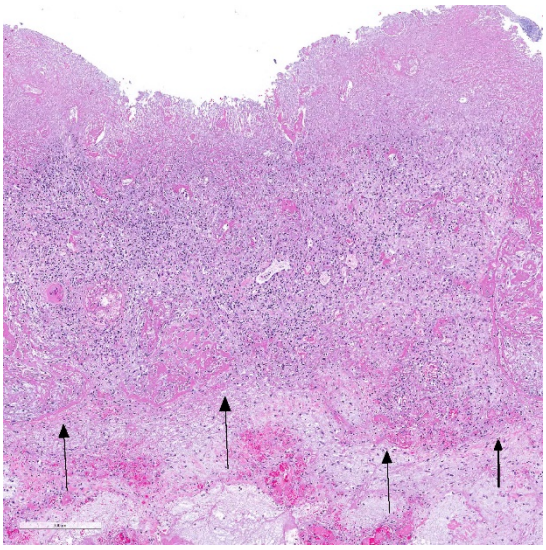
nonsteroidal anti-inflammatory drugs (NSAIDs) toxicity.[3,8]

NSAIDs cause ulceration of the small intestine and colon in horses and other animals. In horses, even low doses of phenylbutazone can cause ischemic damage to the intestinal mucosa.[8] Stress and/or dehydration are considered contributory factors to the pathogenesis of this condition.[8] Because the right dorsal colon is preferentially involved, the condition is usually named *"right dorsal colitis";* however, lesions in other parts of the colon and in the small intestine may also occur.[4,6,7] The pathogenesis of this condition is associated with decreased production of prostaglandin E_2 and nitric oxide. Decreased prostaglandin is due to NSAID inhibition of cyclooxygenase 2 (COX-2).[8]

Morphologically, right dorsal colitis cannot be differentiated from some of the most common infectious colitis of horses (e.g. *C. difficile, C. perfringens* and *Salmonella spp.* infections). There are no specific tests to confirm the diagnosis of NSAID's toxicity, and the diagnosis should therefore be based on a history of NSAIDs administration and ruling out infectious causes of colitis. While location of the lesion in the right dorsal colon helps to establish a diagnosis of NSAIDs toxicity, it should be kept in mind that these drugs can also affect other parts of the alimentary canal in horses.[8]



Right dorsal colon, horse. At subgross magnification, there is diffuse necrosis of the mucosal, profound submucosal edema, and marked congestion with hemorrhage and edema in all layers of the gut wall. (HE, 5X)



Right dorsal colon, horse. At subgross magnification, there is diffuse necrosis of the mucosal, profound submucosal edema, and marked congestion with hemorrhage and edema in all layers of the gut wall. (HE, 5X)

Contributing Institution:

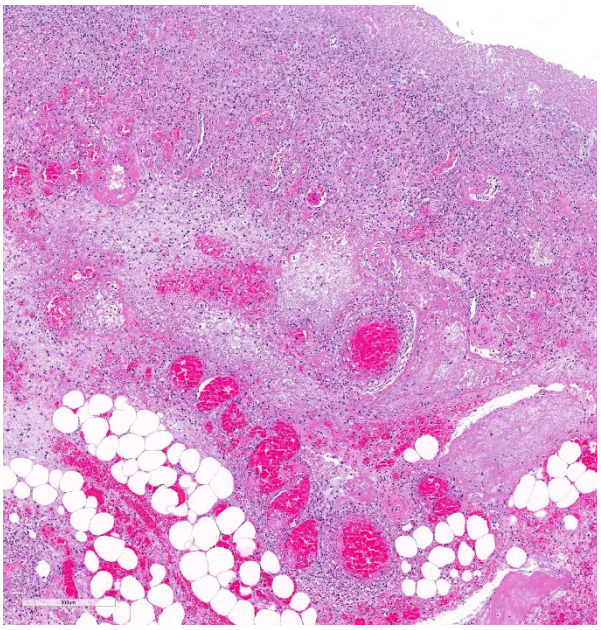
California Animal Health and Food safety Laboratory system, San Bernardino branch, University of California, Davis.

JPC Diagnosis: Colon: Mucosal necrosis, diffuse, with transmural vascular fibrinoid necrosis and thrombosis, and severe submucosal edema.

JPC Comment: Since the mid-1980's, overuse of non-steroidal anti-inflammatory drugs has been associated with a variety of lesions in horses, to include ulceration of various segments of the gastrointestinal tract, as well as papillary necrosis in the kidney and

vascular damage in multiple organs.[2] Hypovolemia has been shown to potentiate their anti-prostaglandin effects, making their usage particularly hazardous in horses with endotoxemia or colic. The published toxic dose for phenylbutazone in the horse is 8.8 mg/kg BW[5] while in hypovolemic animals it is lowered to 6.0 mg/kg BW[1].

Gastrointestinal ulceration may be seen in a number of sites in the GI tract with aggressive NSAID use, to include the mouth, esophagus, and glandular stomach. While the reason for the particular susceptibility of the right dorsal colon is largely unknown, a number of theories have been advanced but not proven. The slow transit time and proximal narrowing as it enters the small colon may allow increased mucosal contact time for orally dosed NSAIDs bound to feed particles. Concentration gradients within the dorsal colon may also play a role, as the dorsal colon differs from the ventral colon in that fluid may be secreted as well as absorbed.[1]



Right dorsal colon, horse. There is fibrinoid necrosis of vessels of all sizes in the mucosa and submucosa (black arrows). The submucosal adipose tissue is a good marker for colon in the horse in sections in which mucosa is totally necrotic. (HE, 83X)

Both acute and chronic syndromes of right dorsal colitis have been described, with horses with acute disease manifesting with colic, diarrhea, fever, depression, and shock. More chronic disease results in intermittent colic with weight loss, soft but not diarrheic feces and ventral edema. Hypoproteinemia and hypoalbuminemia are commonly seen in both acute and chronic presentations, with more severe levels and panhypoproteinemia seen in cases with more chronic courses.[2]

At necropsy, areas of ulceration may be interrupted by islands of regenerating mucosa, and in chronic cases, colonic submucosal fibrosis, stenosis and impaction may be evident.[2]

References:

1. Galvin N, Dillon H, McGovern F. Right dorsal colitis in the horse: minimreview and reports on three cases in Ireland. *Irish Vet J* 2004; 57(8): 467-473.
2. Karcher LF, Gill SG, Anderson WI, King JM. Right dorsal colitis. *J Vet Int Med* 1990; 4:247-253.
3. Jones SL, Davis J, Rowlingson K. 2003. Ultrasonographic findings in horses with right dorsal colitis: five cases (2000–2001*). J Am Vet Med Assoc* 222:1248–1251.
4. Marshall JF, Blikslager AT. 2011. The effect of nonsteroidal anti-inflammatory drugs on the equine intestine. *Equine Vet J* Suppl 39:140-144.
5. Meschter CL, Gilbert M, Krook L, Maylin G, Corradino R. The effects of phenylbutaxone of the morphology

of prostaganding concentras of the pyloric mucosa f the equine stomach. *Vet Pathol* 1990; 27:244-253.
6. Parfitt JR, Driman DK. 2007. Pathological effects of drugs on the gastrointestinal tract: a review. *Hum Pathol* 38:527-536.
7. Rotting AK, Freeman DE, Constable PD, Eurell JA, Wallig MA. 2004. Effects of phenylbutazone, indomethacin, prostaglandin E_2, butyrate, and glutamine on restitution of oxidant-injured right dorsal colon of horses in vitro. *Am J Vet Res* 65:1589–1595.
8. Uzal FA, Plattner BL, Hostetter JM. Alimentary system. 2016. In: Maxie G ed., Jubb, Kennedy and Palmer's Pathology of domestic animals. Vol. 2, pp. 1-257. Elsevier, St. Louis, MO.

CASE IV: WSC Case 2 (DVD) (JPC 4083951). Tissue from a horse (*Equus caballus*).

Signalment: 26-year-old male horse (*Equus ferus caballus*), breed not documented

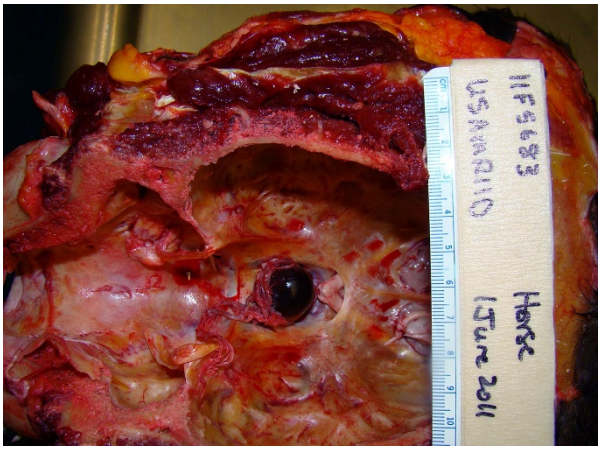
History: This horse was part of a protocol for which blood samples were collected periodically to provide blood and/or blood products for use in other research projects. The horse was ataxic, lethargic, and exhibited reported neurologic signs for approximately 2 days prior to being euthanized.

This horse was part of a research project conducted under an IACUC approved protocol in compliance with the Animal Welfare Act, PHS Policy, and other federal statutes and regulations relating to animals and experiments involving animals. The facility where this research was conducted is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International and adheres to principles stated in the 8th edition of the Guide for the Care and Use of Laboratory Animals, National Research Council, 2011.

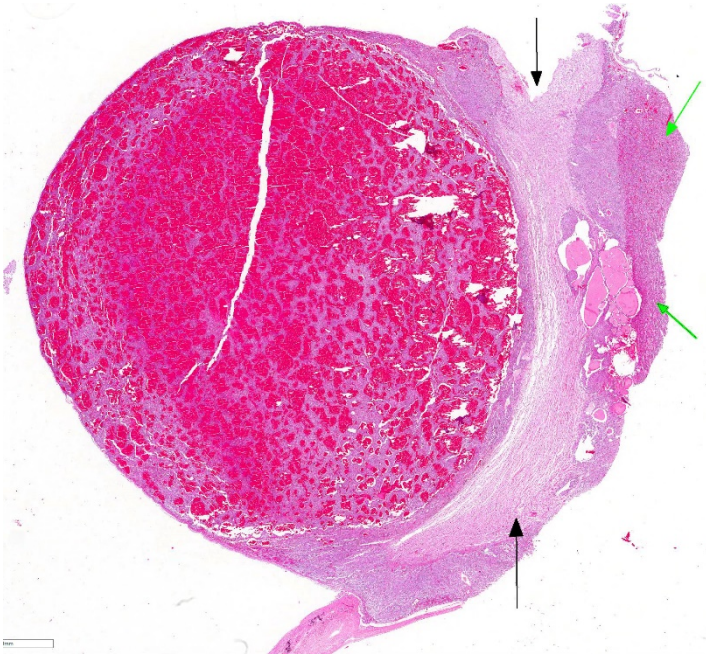
Gross Pathology: Due to extenuating circumstances and adverse weather conditions, the only portion of this animal harvested for necropsy was the head. Because the horse exhibited neurologic signs prior to euthanasia, both rabies and herpes tests were performed on postmortem brain samples at the local diagnostic laboratory, and were negative. Upon gross examination of the brain, it was noted that the pituitary gland was dark red, soft, and enlarged, measuring 2.5 x 2.5 x 3 cm.

Laboratory results: Rabies and herpes tests: Negative.

Microscopic Description: Pituitary gland: Expanding the pars intermedia, compressing adjacent tissue, is a well-circumscribed,



Pituitary gland in situ, horse. The pituitary gland extends upward out of the sella turcica. (Photo courtesy of : USAMRIID, Pathology Division, 1425 Porter Street, Frederick, MD 21702-5011, http://www.usamriid.army.mil/)



Pituitary gland, horse. A 1.75cm nodule arising from the pars intermedia, compressing the adjacent pars nervosa (black arrow) and pars glandularis (green arrow). (HE, 8X)
)

partially encapsulated, moderately cellular neoplasm composed of polygonal cells arranged in nests and packets supported by a fine fibrovascular stroma. Neoplastic cells have indistinct cell borders, moderate amounts of granular, often microvacuolated eosinophilic cytoplasm, a round, antibasilar nucleus with finely stippled chromatin, and up to 3 variably distinct nucleoli. Mitosis average less than one per 10 HPFs. Anisocytosis and anisokaryosis are mild to moderate. Diffusely throughout the neoplasm, neoplastic cells often surround variably sized and shaped blood-filled cystic spaces and occasionally palisade around blood vessels, forming pseudorosettes. Neoplastic cells occasionally form follicles lined by low cuboidal cells and filled with homogenous eosinophilic material (colloid) and cellular debris. Occasionally, scattered throughout the neoplasm and adjacent pars nervosa, there are globules of gold-brown pigment (hemosiderin) and bright yellow pigment (hematoidin) accompanied by hemosiderin-laden macrophages. There is multifocal, minimal hemorrhage.

Contributor's Morphologic Diagnosis:

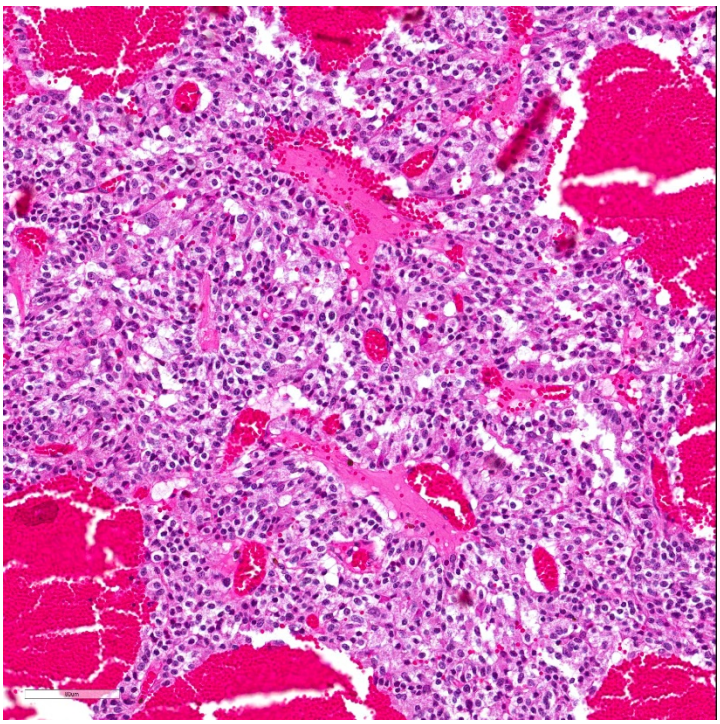
Pituitary gland, pars intermedia: Adenoma, breed unspecified, equine.

Contributor's Comment: Adenoma of the pars intermedia is the most common pituitary tumor in the horse.[1] Grossly, the tumors are well circumscribed, partially encapsulated, multinodular, space occupying lesions that tend to expand, and subsequently compress, the overlying hypothalamus.[1] Microscopically, neoplastic cells are polygonal to spindle shaped with eosinophilic, granular cytoplasm, and an oval, hyperchromatic nucleus. Neoplastic cells are arranged in nodules, rosettes, bundles, or follicular structures separated by fine, fibrovascular septa, often containing numerous capillaries.[1,4]

Secondary compression of the hypothalamus can greatly diminish its normal function, resulting in the clinical syndrome associated with the tumor, termed Pituitary Pars Intermedia Dysfunction (PPID). PPID is one of the most commonly diagnosed equine endocrinopathies.[7] PPID was originally imprecisely termed "equine Cushing's disease," due to its similarities to human Cushing's disease. However, in human Cushing's disease, the pars distalis, rather than the pars intermedia, is affected. Additionally, in humans, the condition is

usually not neoplastic, and exhibits more adrenal gland involvement.[5] Since the hypothalamus is responsible for regulating body temperature, appetite, and cyclic shedding of hair, clinical signs in the horse include hyperhidrosis, polyphagia, hirsutism, polyuria, polydipsia, laminitis, muscle atrophy, fat accumulation, lethargy, and some metabolic abnormalities, among others.[1,5,6,7] Hypothalamus compression and resultant PPID is not limited in causation to neoplasia, and can also result from pituitary hypertrophy and hyperplasia, with the pituitary gland enlarging 2 to 5 times its normal weight.[5]

There are conflicting reports whether there is a gender predisposition in horses with PPID. Historically, literature has reported that female horses are more frequently affected than males, while others have noted a weak association with gelding sex.[4] Still other studies report no sex differences in the risk for developing PPID, including a review with meta-analysis of 6 studies.[3] However, there is overall consensus that PPID is a chronic progressive disease that overwhelmingly affects older horses, and is considered one of the most common diseases of horses 15 years of age and older.[5,6] The population of aged horses has considerably increased within the past two decades, and clients are becoming more informed and aware of equine age-associated diseases.[5] A recent report that investigated disease prevalence in older horses found that pituitary pars intermedia adenoma was the most common neoplasm in horses 15 years of age and older, and that PPID was the most common specific diagnosis.[6] Additionally, when accounting for the reason for euthanasia, PPID was found to be the second-most common cause of death in aged horses, with disease of the digestive system as the first.[6]



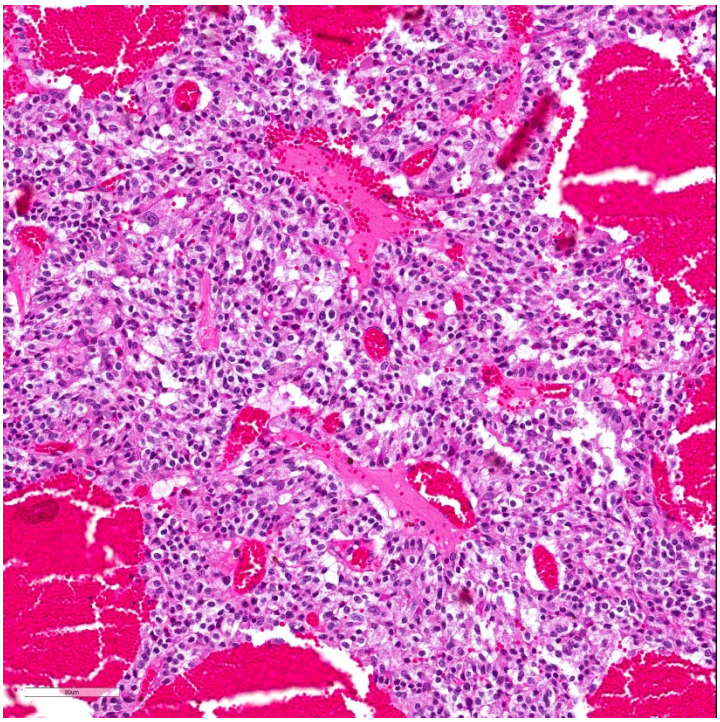
Pituitary gland, horse. Neoplastic cells form vague nests and packets and palisade along blood vessels. Numerous areas of hemorrhage and drop are present throughout the mass. (HE, 274X)

Note: Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the U.S. Army.

Contributing Institution:

USAMRIID
Pathology Division
1425 Porter Street
Frederick, MD 21702-5011
http://www.usamriid.army.mil/

JPC Diagnosis: Pituitary gland, pars intermedia: Adenoma.

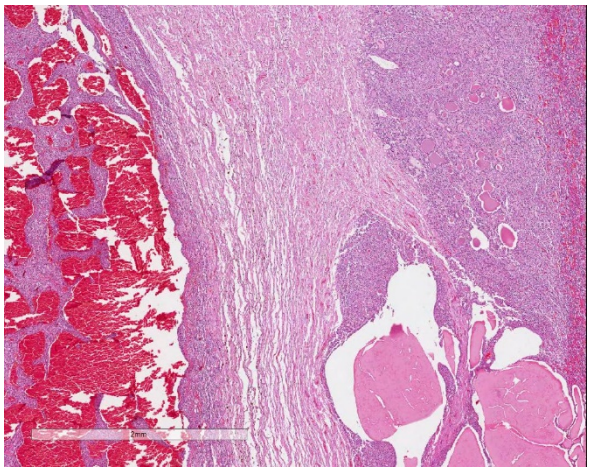


Pituitary gland, horse. Neoplastic cells palisade along vessels, forming pseudorosettes. (HE, 275X)

JPC Comment: The contributor has provided a very concise but thorough review of this particular neoplasm (largely restricted to the dog and horse), as well as the very important and common condition of pars pituitary intermedia disease (PPID). It bears repeating that the two entities are different, in that the pituitary pars intermedia adenoma (PI adenoma) is a common neoplasm in older horses, while PPID is the clinical syndrome arising from the compression of the hypothalamus from neoplasms or hyperplastic lesions of the pituitary. Characteristic clinical signs of PPID in the horse include hirsutism, delayed shedding, polydipsia and polyuria, weight loss, laminitis, and reproductive disorders in mares.[2]

A histologic and stereologic study was performed on 124 horses, and a number of histologic findings were significantly correlated with age, to include follicles and cysts within the pars intermedia, lipofuscin in the pars nervosa, and focal chromophobe hyperplasia in the pars glandularis. Highlighting the difference between adenoma and PPID, in this study, 22/124 horses had adenomas of the pars intermedia, but only 4/124 demonstrated clinical signs associated with PPID. In this study, most PI adenomas were considered incidental findings and non-functional. (Interestingly, in another review of old age lesions in the horse, in a total of 40 horses with PI adenoma, 23 were euthanized because of the pituitary adenoma. Interestingly, stereological review of PI adenomas showed that the neoplastic cells of PI adenomas causing PPID were larger by volume than those in horses without PPID.

Pituitary adenomas may also be part of a constellation of endocrine tumors in horses.



Pituitary gland, horse. Non-neoplastic areas of the pars intermedia (right) contain clusters of colloid-filled follicles. (HE, 275X)

In a recent multicenter study on equine pheochromocytoma, 18/37 (49%) animals had a concurrent PI adenoma, and 8/37 had a third neoplasm (thyroid adenoma, C-cell carcinoma), ultimately resulting in a diagnosis of multiple endocrine neoplasia. In humans, type I multiple endocrine neoplasia (MEN) includes pheochromocytoma and pituitary gland tumors.

References:

1. Capen CC. Endocrine glands. In: Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. Vol 3. 5th ed. Philadelphia, PA: Elsevier Saunders; 2007:326-376.
2. Hatazoe T, Kawaguchi H, Hobo S, Misumi K. Pituitary pars intermedia dysfunction (equine Cushing's disease) in a thoroughbred stallion: a single report. *J Equine Sci* 2015*; 26(4):125-128.*
3. Leitenbacher J, Herbach N. Age-related qualitative histological and quantitative stereological changes in the equine pituitary. *J Comp Path* 2016; 154:215-224.
4. Luethy D, Habecker P, Murphy B, Nolen-Walston R. Clinical and pathological featurs of pheochromocytoma in the horse a multi-center retrospective study of 37 cases (2007-2014). *J Vt Inten Med* 2016; 30:309-313.
5. McFarlane D. Endocrine and metabolic disorders. In: Smith BP, ed. *Large Animal Internal Medicine,* 4th ed. St. Louis, MO: Elsevier; 2009:1340-1344.
6. Miller MA, Moore GE, Bertin FR, Kritchevsky JE. What's new in old horses? Postmortem dignoses in mature and aged equids. *Vet Pathol.* 2016;53(2):390-398.
7. Rohrbach BW, Stafford JR, Clermont RS, et al. Diagnostic frequency, response to therapy, and long-term prognosis among horses and ponies with pituitary par intermedia dysfunction, 1993–2004. J Vet Intern Med. 2012; 26(4):1027–1034.