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Department of Veterinary Pathology
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CONFERENCE 11
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Conference Moderator: MAJ Brett Saladino
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Washington, DC 20306-6000

CASE I – ND2 (AFIP 2791095)

Signalment: Ten-week-old, ring-necked pheasant (*Phasianus colchicus*)

History: Approximately 120 of 1000 ten-week-old ring-necked pheasant chicks being reared on dirt died over a three-day period, whereas barn raised chicks of the same age experienced minimal mortality. Signs in affected chicks were limited to runny yellow stools developing a few hours prior to death.

Gross Pathology: Three dead and one live inactive chick were received in “light” body condition. Macroscopic findings in all included marked hepatosplenitis – disseminated 0.1-0.4 cm diameter yellow-white foci of necrosis/inflammation on capsular and cut surfaces of all livers and enlarged multinodular spleens - and marked thickening of the cecae in all chicks.

Laboratory Results: Bacteriology: Liver: *E. coli*; Intestine: mixed environmental bacteria; *Salmonella* sp. not isolated on enrichment culture

Contributor’s Morphologic Diagnosis: Marked multifocal to coalescing necrotizing to necrogranulomatous hepatosplenitis and typhlitis with intralesional histomonads

Contributor’s Comment: Consistent histologic lesions in all chicks included marked multifocal necrotizing to granulomatous hepatitis, splenitis and typhlitis with high numbers of intralesional protozoal organisms consistent with *Histomonas meleagridis*. Various stages of coccidia were also identified in some sections of intestine. Acid-fast stains were negative on all tissues. Interstitial myocarditis and pneumonitis with intralesional histomonads were also observed in tissues of one chick.

AFIP Diagnoses: 1. Liver: Hepatitis, necrotizing, subacute, multifocal to coalescing, moderate, with fibrinous capsulitis and numerous protozoa, etiology consistent with *Histomonas meleagridis*, Ring-necked pheasant (*Phasianus colchicus*), avian.
2. Cecum: Typhlitis, necrotizing, transmural, subacute, multifocal, moderate, with ulceration, and numerous protozoa.
3. Spleen: Splenitis, necrotizing, subacute, multifocal to coalescing, moderate, with lymphoid depletion and numerous protozoa.

Conference Comment: One of the most intriguing aspects of *Histomonas meleagridis*, a protozoan of the order Trichomonadida, is the intricate life cycle. As free trophozoites passed in the feces, *H. meleagridis* is extremely delicate, lasting only minutes in the environment. Successful transmission of this organism is reliant on the unique relationship with *Heterakis gallinarum*, a cecal nematode. Within the cecum, *H. gallinarum* eggs become infected with *H. meleagridis* trophozoites by an unknown mechanism and are passed in the feces. Protected in the nematode egg, *H. meleagridis* remains viable for extended periods. Earthworms consume infected nematode eggs, further concentrating the organisms in their tissues. Birds become infected with *H. meleagridis* via ingestion of the infected nematode eggs or earthworms.

Once in the cecum, *H. meleagridis* is released from the nematode egg and penetrates the cecal wall becoming very ameboid in appearance. Interestingly, certain bacteria (e.g. *Clostridium perfringens*, *Escherichia coli*) play a role in the development and severity of lesions. The lesion is characterized by lymphohistiocytic inflammation and necrosis which can become transmural. Rupture and subsequent coelomitis is not uncommon. Cecal cores composed of sloughed epithelium, inflammatory cells, fibrinous exudate, and ingesta form in the lumen.

Vascular invasion results in dissemination to the liver. Multifocal scattered portal lesions are visible 6-7 days post infection. Affected areas often coalesce and are characterized by abundant lymphocytes, histiocytes, and heterophils. Extensive necrosis results in the characteristic targetoid lesion seen grossly.

Contributor: North Dakota State University, Veterinary Diagnostic Services Department, Fargo, ND 58105

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2. McDougald L: Other Protozoan Diseases of the Intestinal Tract; Histomoniasis (Blackhead). In: Diseases of Poultry, ed. Calnek BW, 10th ed., pp. 890-895. Iowa State University Press, Ames, IA, 1997

3. McDougald L, Hu J: Blackhead disease (*Histomonas meleagridis*) aggravated in broiler chickens by concurrent infection with cecal coccidiosis (*Eimeria tenella*). *Avian Dis* **45**:307-312, 2001

CASE II – E10233 (AFIP 2787207)

Signalment: 2-days-old, female, Paint (white) foal (*Equus caballus*)

History: The foal appeared normal at birth, failed to pass meconium, and within hours developed progressively severe symptoms of colic. The abdominal pain became more intense and the animal had to be euthanized.

Gross Pathology: The main post-mortem findings in this foal were confined to the digestive tract. The stomach, duodenum and cranial 2/3rds of the jejunum were gas-filled but otherwise unremarkable. The distal jejunum, ileum, cecum and great colon were moderately distended with meconium. The small colon was empty and appeared contracted (the colonic lumen was approximately 3 mm in diameter).

Laboratory Results: None

Contributor's Morphologic Diagnosis: Ileocolonic aganglionosis (lethal white foal syndrome)

Contributor's Comment: Microscopic examination of multiple sections of the ileum and large intestine revealed complete absence of the ganglia of the myenteric (Auerbach's) and submucosal (Meissner's) plexuses. The submitted slide contains sections of the small colon.

Gross and microscopic findings in this foal were consistent with those of congenital ileocolonic aganglionosis (lethal white foal syndrome) reported in the progeny of overo spotted horses. Embryologically, both melanocytes and enteric ganglionic neurons are of neural crest origin. This may explain the association between the presence of unpigmented skin and the lack of enteric ganglia in affected foals.

AFIP Diagnosis: Colon: Aganglionosis, diffuse, Paint (*Equus caballus*), equine.

Conference Comment: The Paint breed of horse is defined by its color pattern; the two most common being overo and tobiano. Overo color patterns are further

subclassified and include frame, splashed white, calico, sabino, bald faced, and medicine hat subtypes. Multiple genes are thought to control the distribution and amount of white in these distinctive patterns.

Endothelin receptor B (EDNRB) along with its ligand endothelin 3 are responsible for the migration and development of neural crest cells. Neural crest cells give rise to melanocytes, chromaffin cells of the adrenal medulla, peripheral nerve sheath cells, and ganglia of the sensory cranial nerves and myenteric plexus.

Overo horses, particularly those of the frame subtype, are often heterozygous with a substitution of lysine for isoleucine at the 118 residue (Ile118) of EDNRB. Although heterozygotes are unaffected, homozygous offspring (Lys118) have defective neural crest cell development. These homozygotes are often born solid white and lack ganglia in the large intestine.

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References: 1. Hultgren B: Ileocolonic aganglionosis in white progeny of overo spotted horses. J Am Vet Med Assoc **180**:289-292, 1982
2. Santschi E, Vrotsos P, Purdy A, Mickelson J: Incidence of the endothelin receptor B mutation that causes lethal white foal syndrome in white -patterned horses. Am J Vet Res **62**:97-103, 2001
3. Trommershausen-Smith A: Lethal white foals in matings of overo spotted horses. Theriogenology **8**:303-311, 1977
4. Vonderfecht S, Trommershausen-Bowling A, Cohen M: Congenital intestinal aganglionosis in white foals. Vet Pathol **20**:65-70, 1983

CASE III – 01-955 (AFIP 2789023)

Signalment: Chinook salmon (*Oncorhynchus tshawytscha*)

History: Four months after introduction into salt water, during a routine fish health check of netpen reared, 200-250 gm Chinook salmon (*Oncorhynchus tshawytscha*), hematology of 2 of 60 fish disclosed a small number of hemoflagellates. The fish had been derived from a mixed ground and surface supplied, flow-through, fresh water hatchery. After introduction to salt water, the stock had a history of low-grade vibriosis (*Listonella anguillarum*) and bacterial kidney disease (*Renibacterium salmoninarum*). Approximately 12 months after initial detection of this parasite, within a 2-3 week span, there was a gradual increase in mortality with a nadir of 3%/week. At that time, the salmon had

attained an average weight of 3.5 kg and the stocking density was 10kg/m³. The fish were maintained on a standard commercial pellet ration.

Clinical signs were uniform throughout the site and included numerous moribund, listless fish lingering around perimeter of the pens, dark discoloration, anorexia, and rapid, repetitive flaring of the operculi (tachypnea).

Gross Pathology: Gross examination disclosed generalized anemia (blanched gills), bilateral exophthalmia, raised scales (subcutaneous edema), and serosanguinous ascites. The livers were pale and dark green brown (biliary stasis) and the kidneys were grey black, swollen and firm to friable. The spleens were massively enlarged, occupying up to one half to two thirds the volume of the coelomic cavity, firm, glistening and homogeneous red black. In most fish the stomachs were empty, the mucosa was diffusely stained yellow green and the lower intestine contained bright yellow casts. The gall bladders were distended and turgid. Hematocrits of multiple fish were between 10-20% (normal >40%) and the blood was watery.

Laboratory Results: None

Contributor's Morphologic Diagnosis: Vasculature, multisystemic: Leukocytosis, moderate, multifocal, mononuclear and neutrophilic, with occasional melanin-laden macrophages and variable numbers of hemoflagellated protozoa compatible with *Cryptobia salmonisitica*.

Contributor's Comment: Multisystemic: The lumina of multiple small to intermediate caliber, thin walled blood vessels are mildly to moderately dilated and contain variable numbers and concentrations of extracellular, 10-20x2-6 μ m, biflagelated protozoa, admixed with scant to moderate amounts of refractile, homogeneous to finely granular eosinophilic deposits, erythrocytes, fewer mononuclear cells, neutrophils, and occasional melanin laden macrophages. Within the renal sinusoids, there is multifocal congestion and hyperemia with occasional erythrophagocytosis, dispersed melanin granules and scattered protozoa. The intervening hematopoietic tissue is mildly to moderately hyperplastic with a preponderance of maturing lymphomyeloid cells. The glomeruli feature segmental to diffuse expansion of the mesangium by dense, homogeneous material.

The gross and microscopic findings are compatible with cryptobiosis (*Cryptobia salmonisitica*), which would have been sufficiently severe to account for the increased morbidity and mortality of the stock. This parasite has been reported in virtually all species of Pacific salmon along the western seaboard of North America from California through southern British Columbia and southwest Alaska. To date, cryptobiosis has been recognized in 8 species of salmon, 7 species of sculpin, a sucker, and the three spined stickleback. A survey of Pacific salmon throughout the west coast of British Columbia revealed that this parasite was

confined to tributaries of the Fraser River, adjoining coastal rivers, and select rivers on Vancouver Island. Cryptobiosis has been reported in both wild and farmed fish stocks with experimental challenges of Chinook (*Oncorhynchus tshawytscha*) and Coho (*O. kisutch*) salmon resulting in 100% and 0% mortality, respectively. This case is believed to be one of the first epizootics of this condition in farmed Pacific salmon along the BC coast. Infection can result in ascites, anemia (microcytic and hypochromic), exophthalmia, splenomegaly, anorexia and profound immunosuppression. Transmission is accomplished by a leech vector (*Piscicola salmositica*) as well as horizontal dissemination between susceptible fish. In this case, additional tissues were collected and forwarded to an outside reference lab; sera from clinically affected and apparently normal fish were positive by ELISA and naïve fish vaccinated for *C. salmositica* were protected against infection by routine challenge exposure. Management included immediate harvest of affected fish. Because wild fish are the probable source of infection and the hatchery was considered the likely point source of exposure, this facility has since been modified so that the water supply is solely a ground source. The glomerular alterations may represent a sequela to cryptobiosis or possibly a past infection with bacterial kidney disease (*R. salmoninarum*), which has been reported to induce glomerular alterations in rainbow trout.

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- AFIP Diagnoses:**
1. Multiple tissues: Intravascular and extravascular flagellated protozoa, etiology consistent with *Cryptobia salmositica*, Chinook salmon (*Oncorhynchus tshawytscha*), piscine.
 2. Perirenal fibroadipose tissue: Inflammation, granulocytic and histiocytic, subacute, with myriad flagellated protozoa.
 3. Kidney, hematopoietic tissue: Hyperplasia, lymphomyeloid, moderate.

Conference Comment: *Cryptobia salmositica* is a biflagellate protozoan of the order Kinetoplastida. Both flagella originate on the anterior end of the organism. The first is free; the second is attached to, and runs along the body wall ending as a free flagella on the posterior end. The prevalence and intensity of the infection is seasonal, coinciding with the return of adult salmon to fresh water in the autumn and an increase in the leech vector, *Piscicola salmositica*. Infection is also reported by direct transmission in experimental and hatchery conditions.

Virulence of the organism is attributed to cysteine proteases and metalloproteases; both have activity against hemoglobin, fibrinogen, gelatin, and albumin. Metalloprotease is erythrolytic and collagenolytic, degrading collagen types I, IV, and V.

Control strategies include vaccinating which provides control via the production of complement fixing antibodies, enhanced phagocytosis, and cell mediated cytotoxicity. Exploitation of innate immunity with crossbreeding to resistant and tolerant fish species is an area of current research. Cryptobia-

resistant charr prevent infection by lysing parasites via activation of the alternate complement pathway. Cryptobia-tolerant charr are susceptible to infection but neutralize the harmful metalloproteases with release of alpha2-macroglobulin, a natural antiprotease.

Contributor: British Columbia Ministry of Agriculture, Animal Health Center, Abbotsford, BC, Canada V3G 2M3

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CASE IV – 00-7295 (AFIP 2754164)

Signalment: 4-week-old, male, crossbred pig (*Sus scrofa*)

History: The piglet was from a university swine herd. Piglets were born healthy and began developing skin lesions and weight loss 5-6 days prior to necropsy. All piglets in the litter were affected. Recent management changes included new farrowing pens in which the barrier wall allowing piglets access to creep feed contained sharp metal edges that caused skin abrasions to the growing piglets. Affected piglets were treated with tetracycline and Tylan. This piglet died and was submitted for necropsy.

Gross Pathology: The piglet was in adequate body condition. Multifocal to coalescing crusted skin lesions were present affecting all parts of the body. Lesions were approximately 0.5-2.5 cm diameter, slightly raised, and had brown friable surfaces. Fibrinous adhesions of the cranial lung lobes to the thoracic wall were found, with associated moderate pleural thickening, but lung parenchyma was grossly normal. No gross lesions were found in other organs.

Laboratory Results: Specimens of skin, lung, and liver were submitted for aerobic bacterial culture. A heavy growth of *Staphylococcus hyicus* was obtained from the skin, and this organism was cultured in low numbers from the lung and liver.

Contributor's Morphologic Diagnosis: Skin: Severe chronic pustular epidermitis and superficial folliculitis due to *Staphylococcus hyicus* (exudative epidermitis; "greasy pig disease")

Contributor's Comment: Skin sections exhibit marked epidermal acanthosis with large coalescing zones of superficial epidermal necrosis and neutrophilic infiltration. Necrosis and suppurative inflammation extend into superficial follicles and there is suppurative adenitis in some areas (may not be present on every slide). Neutrophils are degenerate and are admixed with a large number of bacterial cocci. There is increased vascularity of the superficial dermis with a small number of perivascular to interstitial lymphocytes with a smaller number of admixed neutrophils. There is occasional rupture of affected follicles. The bacterial organisms stained positively with gram stain. A locally extensive zone of fibrinosuppurative pleuritis was present in the area of fibrinous adhesion to the thoracic wall, and no significant lesions were found in the liver.

Exudative epidermitis due to *Staphylococcus hyicus* is a worldwide problem in piglets. The organism can often be isolated from the mucosa and skin of healthy adult pigs, and can persist in the environment for long periods. Disease occurs only in young piglets up to about 35 days of age. Passive transfer of antibodies from immune sows and development of immunity with age appear to adequately protect against disease. Piglets from non-immune sows are predisposed. Skin trauma, such as due to fighting, allowing entry of infective organisms is also considered a risk factor in this disease.

Staphylococcus hyicus is a gram-positive coccus present in large numbers in skin lesions of affected pigs. *Staphylococcus hyicus* produces an exfoliative toxin of approximately 30 kDa that causes separation of cells in the upper stratum spinosum resulting in rapid intraepidermal spread of organisms. Death of affected piglets is common and is attributed to dehydration, septicemia, or both. This porcine disorder has been likened to "scalded skin syndrome" in human neonates, due to skin infection by exfoliative toxin-producing *Staphylococcus aureus*. Exudative epidermitis had not occurred in this facility prior to the introduction of the new farrowing pens. Infection in this litter was suspected to have occurred secondary to skin abrasions occurring due to the sharp metal points in the pen. No new cases have appeared in the year following removal of the sharp points.

AFIP Diagnosis: Haired skin: Epidermitis and infundibular folliculitis, exudative and proliferative, subacute, diffuse, moderate, with multifocal ulceration, superficial dermatitis, and myriad cocci, crossbred pig (*Sus scrofa*), porcine.

Conference Comment: The contributor has provided a concise review of the important aspects of *Staphylococcus hyicus*.

The differential diagnosis discussed in conference included mange (*Sarcoptes scabiei* var. *suis*), swine parakeratosis (zinc and essential fatty acid deficiency), porcine juvenile pustular psoriaform dermatitis (collarettes or rings typically on the

ventrum of young pigs), dermatosis vegetans (associated with a giant cell pneumonia), and ringworm (most commonly *Microsporum nanum*).

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