

**The Armed Forces Institute of Pathology
Department of Veterinary Pathology
WEDNESDAY SLIDE CONFERENCE
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**CONFERENCE 16
5 March 2003**

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CASE I – 02-989F (AFIP 2840408)

Signalment: Cultured Sea Bream (*Sparus aurata*), weight >400g (more than 1-year-old)

History: In the affected tanks, there was anorexia, weight loss with emaciation, and then cachexia, and increased mortality ($\pm 1\text{p}100$ per week).

Gross Pathology: Cachexia, distended abdomen, no macroscopic intestinal lesions identified

Laboratory Results: Protozoan parasites on fresh smears from the rectal part of the digestive tract

Contributor's Morphologic Diagnosis: Rectum: Mild to severe diffuse enteritis associated with severe generalized infection of the rectal epithelium with different stages of PAS positive disporic plasmodia identified as *Myxidium leei*: Severe rectal myxidiosis

Contributor's Comment: *Myxidium leei* is a myxozoan parasite of cultured fishes in the Mediterranean areas. The infection is especially observed in Sparids: sea bream, *Sparus aurata*, sharpnout sea bream, *Puntazzo puntazzo* and red sea bream, *Pagrus major*. The parasites are also observed in various aquarium reared Mediterranean fish species. The most early and heavy infection is seen in the rectum but the parasites may extend to other parts of the digestive tract: the posterior and anterior intestines, the stomach and the epithelium of the gall bladder.

The disease causes important economic losses in cultured Sparids because of increased mortality, growth retardation and cachexia. The parasite source and cycle are not totally elucidated, the tank infection starts with the introduction of infected fish and direct fish to fish transmission is experimentally demonstrated. Infection happens during summer with water temperature more than 22°C but is followed with a very long

incubation period (3/4months). Clinical signs and mortality appear when intestinal epithelium is heavily infected. Reservoir and intermediate hosts are highly possible but not yet demonstrated.

AFIP Diagnosis: Intestine: Enteritis, lymphocytic, diffuse, mild, with intraepithelial protozoa, Sea Bream (*Sparus aurata*), piscine.

Conference Comment: Myxozoans are distinguished from other protozoa by the presence of polar capsules and valved multicellular spores during their life cycle. Use of periodic acid Schiff (PAS) or Giemsa helps to demonstrate the polar capsules, while acid-fast stains demonstrate the mature spores.

Other economically important myxozoans that produce disease in fish include: *Myxobolus cerebralis*, the causative agent of whirling disease in young salmonids, induces necrosis and lysis of the cartilage of the head and vertebrae which results in permanent cranial and skeletal deformities; *Kudoa thysites*, produces myoliquefaction in pen-reared Atlantic salmon; and several myxozoan species which cause disease in pen-reared sea bass and sea bream in the countries near the Mediterranean Sea.

Conference participants included coccidiosis (*Eimeria* spp.) and cryptosporidiosis in their differential diagnosis. Sections may vary in the extent and severity of enteritis.

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CASE II – S1344/02 (AFIP 2839066)

Signalment: 3-year-old, female, Giant Schnauzer, canine

History: The dog had been kept on a farm as a watchdog and liked to drink puddle water. The animal had bloody diarrhea for five months that was unresponsive to

treatment with antibiotics and cortisone. Progressively, bilateral visual impairment and hearing deficit developed. The animal was euthanized.

Gross Pathology: Multiple white nodules of up to 4 mm in diameter were found scattered throughout the cortex and medulla of both kidneys and in the myocardium. Mesenteric lymph nodes were markedly swollen and multiple grayish-white nodules of up to 1.5 cm in diameter were present in the mesenterium and mesometrium. The wall of the colon and especially the mucosa of the colon were thickened and revealed multiple ulcers. Incomplete retinal detachment and several subretinal granulomatous foci were detected in both eyes.

Laboratory Results: *Prototheca* sp. was cultured from the urine, the feces, and CSF of the dog.

Contributor's Morphologic Diagnoses: 1. Heart: Myocarditis, granulomatous, multifocal to coalescing, moderate, with myriad intralesional protothecal organisms, giant schnauzer, canine.
2. Colon: Colitis, pyogranulomatous and ulcerative, diffuse, severe, with myriads of intralesional protothecal organisms, giant schnauzer, canine.

Contributor's Comment: This is a typical case of canine disseminated protothecosis. Interspersed within the myocardium there are several granulomas with central accumulation of protothecal organisms surrounded by macrophages, plasma cells, and lymphocytes. The algal organisms are round to oval, 5-20 um in diameter, and have a thin, but distinct cell wall. The organisms have a round basophilic nucleus that contains a small conspicuous nucleolus. Multifocally, due to endosporulation, large parent cells (sporangia) contain numerous smaller progeny cells, each surrounded by its own cell wall. Protothecal organisms are present extracellularly or phagocytosed within macrophages. Myofibers within and adjacent to the granulomas multifocally undergo necrosis. The wall of the colon is thickened due to multifocal to diffuse pyogranulomatous inflammation and granulation tissue formation within the mucosa and submucosa with myriad protothecal organisms. Inflammation multifocally extends into the tunica muscularis and subserosa and protothecal organisms are present multifocally within these layers. The mucosa is focally ulcerated. Granulomas within the mucosa and submucosa often reveal central necrosis and fibrin accumulation. Besides the lesions in the colon and myocardium, granulomatous foci with protothecal organisms were observed in the small intestine, kidneys, mesenterium, mesometrium, mammary gland, central nervous system, both eyes, and cochlea of the inner ear.

Protothecosis is a relatively rare disease of animals and men that is caused by saprophytic achlorophyllus algae of the genus *Prototheca*. Within the genus, *P. zopfii* and *P. wickerhamii* are known to cause local or systemic infections in immunocompromised hosts. In dogs disseminated infection prevails and protothecal organisms can be detected within multiple organs. Affected dogs usually show bloody diarrhea due to granulomatous colitis and enteritis, polyuria, loss of weight and debility. In more than 50% of cases, visual impairment or blindness due to uni- or bilateral granulomatous chorioretinitis develops and often acute deafness due to infection of the inner ear and the brainstem is reported. Primary neurologic symptoms are found in

approximately 40% of cases. In dogs the colon seems to be the primary location of invasion with subsequent lymphohematogenic dissemination. There is an obvious discrepancy between the number of protothecal organisms and the degree of inflammatory response that is supposed to be caused by inhibition of migration and proliferation of cellular inflammatory infiltrate by the living infective organism. In contrast, dead protothecal organisms seem to induce an effective immune response.

Protothecal organisms can be stained in tissue sections with periodic acid-Schiff reaction or by Gomori's methenamine-silver staining. The organisms are culturable on Sabouraud-agar and species diagnosis can be made by assimilation tests or fluorescent antibody technique.

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- AFIP Diagnoses:** 1. Intestine: Enteritis, pyogranulomatous, diffuse, severe, with algal organisms, Giant Schnauzer, canine.
2. Heart: Myocarditis, pyogranulomatous, multifocal, mild, with algal organisms.

Conference Comment: The contributor has provided an excellent summary of canine disseminated protothecosis. Protothecosis is most often reported in dogs, cats, cattle and humans. The two pathogenic species are *Prototheca zopfii* and *P. wickerhamii*. *P. zopfii* is the most common etiology of canine ocular protothecosis, causing pyogranulomatous inflammation of the choroid, retina, and subretinal space with subsequent retinal detachment and blindness. *P. zopfii* causes sporadic pyogranulomatous mastitis in cattle, usually on dairy farms where the environment is wet and contaminated with manure, suggesting an ascending route of infection. In cats and humans, mucocutaneous protothecosis is a rare infection caused by *P. wickerhamii*, resulting in nodular to diffuse granulomatous dermatitis and panniculitis.

Sections vary, and may include focal arteritis with thrombosis and algal organisms.

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CASE III – 401-051 (AFIP 2839029)

Signalment: 12 month-old, female, Sprague Dawley rat, *Rattus novegicus*

History: Light microscopic evaluation was performed on selected tissues from male and female Sprague-Dawley rats that had received a control diet, negative control diet, or diets containing various concentrations of ground flaxseed for eight weeks. Differences in incidence or severity of lesions that were considered treatment related were not noted among controls, negative control or treated groups of either sex. The few lesions noted in animals from various groups were consistent with those commonly seen spontaneously in rats of this strain and age. All were considered incidental findings unrelated to treatment.

Gross Pathology: Subcutaneous mass, mammary gland.

Laboratory Results: None.

Contributor's Morphologic Diagnosis: Fibroadenoma, mammary gland.

Contributor's Comment: Presented with a nodular subcutaneous mass that arises in the proximity of very scant amounts of normal mammary gland adjacent to haired skin. The mass is comprised of mainly alveolar and some ductular epithelium arranged in small lobules surrounded and separated by variously dense fibro-vascular connective tissue. Most alveoli consist of a single epithelial cell layer, are slightly dilated and some contain various amounts of an eosinophilic to amphophilic luminal secretion. Alterations of individual alveolar epithelial cells range from cytoplasmic vacuolation, cell swelling, cell death (pyknosis, karyorrhexis) to cell drop out. Infrequent mitotic figures are observed. Alveoli within the lobules are separated by scant, less dense fibro-vascular stroma, while broad bands of dense connective tissue separate and replace the glandular lobules aforementioned. The cellularity and cell morphology of fibroblasts producing the fibro-vascular connective varies within the section. In most areas, the cellularity of the stroma is relatively high and many fibrocytes present blast-like and pleomorphic, with abundant basophilic cytoplasm and occasional atypical mitotic figures. In some peripheral areas, the stroma appears more compressed with lower cellularity and spindeloid shaped fibrocytes. Scant numbers of eosinophils and mast cells are scattered throughout the stroma. The epidermal layer of the overlaying skin appears thin with orthokeratotic hyperkeratosis of the keratin layer.

The fibroadenoma of the mammary gland is one of the most common incidental neoplasms in various rat strains. It is composed of ductular and alveolar epithelium surrounded by variously dense fibrous connective tissue. Sub-classifications into 'alveolar', 'fissured', 'sclerosing' or 'proliferating' fibroadenoma attempt to quantify the

epithelium-to-stroma ratio in a given section. However, the relative proportion of these components varies greatly within the same neoplasm. In addition, the size of the primary tumor may influence the relative amount of stroma more than intrinsic differences of the tissue components since the proliferating connective tissue appears to eventually destroy the glandular tissue.

AFIP Diagnosis: Mammary gland: Fibroadenoma, Sprague-Dawley rat (*Rattus norvegicus*), rodent.

Conference Comment: Conference participants discussed proliferative lesions in rat and mouse mammary glands. In rats, there is variable incidence of mammary neoplasia between strains, and an increased incidence with age. Male rats may spontaneously develop mammary neoplasia, although much less frequently than females. Fibroadenoma is generally considered end stage, not progressing to malignancy. Malignant mammary gland neoplasms of the rat include adenocarcinoma and carcinosarcoma. Adenocarcinoma is uncommon, and may arise within fibroadenoma or adenoma. Carcinosarcoma is rare and is composed of both malignant epithelial and stromal cellular components.

In mice, carcinomas are the most common mammary gland neoplasm, while fibroadenomas occasionally occur. Murine mammary tumor virus (MMTV), an endogenous retrovirus of mice, is transmitted in milk or saliva to offspring. MMTV induces mammary gland adenomas.

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CASE IV – L0105261 (AFIP 2839767)

Signalment: Juvenile (1.8 kg) male New Zealand White rabbit (*Oryctolagus cuniculus*)

History: This rabbit was part of a research study utilizing the isolated ileal loop model to investigate bacterial pathogens. At the time of the initial surgery, the distal ileum was markedly thickened (although no signs of diarrhea or other clinically-evident abnormalities had been noted prior to surgery). The decision was made to complete the short-term study, regardless, and the ileum and terminal jejunum were divided into segments by ligation, and various concentrations of live *Vibrio cholerae* bacteria were injected into the closed loops. Ligated loops were harvested eight hours later, and portions of the remaining (uninoculated) distal ileum and distal jejunum were submitted for histopathological examination.

Gross Pathology: The most distal segment of ileum, and a segment of distal jejunum, both pieces approximately 3 cm long, were submitted in 10% BNF. The ileal mucosa was grossly thickened and had a subtle “cobblestone” appearance. The jejunal mucosa was mildly thickened.

Laboratory Results: No cultures were submitted with the intestinal biopsies.

Contributor's Morphologic Diagnosis: Ileum and jejunum: Enteritis, proliferative and histiocytic, chronic, moderate to marked, diffuse, with intracellular bacteria.

Contributor's Comment: Submitted slides contain sections from both the distal ileum and distal jejunum, but since the lesion in the ileum is more severe, the description will focus on this tissue. The mucosa is diffusely and markedly expanded by an inflammatory cell infiltrate that dissects between and widely separates hyperplastic crypts. The infiltrate consists primarily of large histiocytic cells with eccentric oval nuclei and abundant foamy to granular eosinophilic cytoplasm, admixed with fewer plasma cells, lymphocytes, heterophils and rare multinucleated giant cells. Villi are distinctly blunted and occasionally fused. Tall, often piled-up epithelial cells with increased mitotic figures line most crypts. Scattered crypts are dilated and contain luminal pyknotic cell debris. Acid-fast stains were negative while Warthin-Starry silver stains revealed clusters of small (less than 2 micron), curved- to spiral-shaped organisms within the apical cytoplasm of most crypt epithelial cells. Under oil immersion, these organisms resemble the obligate intracellular bacterium *Lawsonia intracellularis*.

Proliferative enteropathies have been observed in pigs and hamsters since the early half of the 20th century. These diseases were originally thought to be due to a variety of *Campylobacter* spp. that was often cultured from the gut of affected animals. However, in the early 90's, the specific intracellular organism responsible for the lesions was identified and named *Lawsonia intracellularis*. Since then, numerous species (including rabbits) with proliferative enteropathies have been reported and positive identifications of *L.intracellularis* as the causative agent have been made, either by immunohistochemistry, tissue polymerase chain reaction, ribosomal DNA sequencing, *in vitro* culture, silver staining or electron microscopy.

The lesion in this rabbit consisted primarily of a proliferative and histiocytic enteritis, yet some sections also contained areas of superficial erosion, necrosis, submucosal edema and inflammation, and scattered fibrinopurulent serositis. A similar spectrum of lesions can also be seen in other species with intracellular *Lawsonia* infection, (i.e., pigs have four specific syndromes attributed to *Lawsonia* infection and

the reader is referred to the excellent review by Lawson and Gebart, 2000, for more details).

In general, the organisms are found in the apical surfaces of most proliferating epithelial cells of the diseased mucosa, typically the distal ileum, and occasionally the lower bowel. It is interesting to note that while a similar inflammatory infiltrate is present in all sections of jejunum and ileum in this rabbit, virtually no silver-stained organisms were detected in the epithelial cells of the jejunum.

Despite the lack of clinical signs in this rabbit prior to surgery, the pre-existing lesion did compromise the research data, showing that this organism has the potential to interfere with studies if subclinically infected rabbits are inadvertently used.

AFIP Diagnoses: 1. Small intestine: Enteritis, proliferative, histiocytic, diffuse, moderate, with villous blunting and fusion, and multifocal erosion, New Zealand White rabbit (*Oryctolagus cuniculus*), lagomorph.

2. Serosa, mesentery: Peritonitis, suppurative, multifocal, moderate, with plant material.

Conference Comment: Proliferative enteropathy is most common in the pig and hamster, but has been reported in many other species, including some bird species. In the pig, there are four forms of *Lawsonia intracellularis*- induced proliferative enteropathy: porcine intestinal adenomatosis (PIA), proliferative hemorrhagic enteropathy (PHE), necrotic enteritis (NE), and regional ileitis (RI). PIA is a persistent disease of weaner pigs that is grossly characterized by uniform thickening of the mucosa of the ileum and less often, the colon. PHE is an acute disease of pigs older than four months where there is intestinal hemorrhage and less severe mucosal thickening of the ileum than in PIA. NE is a postweaning, often fatal disease with extensive coagulative necrosis of the epithelium. RI is believed to occur in pigs that survive NE, resulting in granulation tissue replacement of the mucosa and hypertrophy of the tunica muscularis.

In mice, *Citrobacter rodentium* causes the rare syndrome of transmissible murine colonic hyperplasia. Unlike *L. intracellularis*, *C. rodentium* bacterial colonies are extracellular, surface-associated organisms. *C. rodentium* induces mucosal hyperplasia that results in weight loss, runting, rectal prolapse, and low mortality.

Sections vary; some may include occasional abscessation of the serosa.

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