The Armed Forces Institute of Pathology
Department of Veterinary Pathology

Conference Coordinator
Matthew Wegner, DVM

WEDNESDAY SLIDE CONFERENCE 2010-2011

Conference 13

8 December 2010

Conference Moderator:
Tim Walsh, DVM, Diplomate ACVP

CASE I: 10-4242 / 10-6076 (AFIP 3170327).

Signalment: 30 Sydney rock oysters, QX resistant broodstock, (*Saccostrea glomerata*).

History: 100% of oysters were at risk with 25% sick and 75% mortality.

Gross Pathology: The oysters varied in size (53.6 ± 8.9 mm shell height). They were in poor condition with minimal gonadal development (1.6 ± 0.9 on a 1-5 scale) and pale digestive glands (1.9 ± 0.9 on a 1-3 scale). On close examination, several of the oysters appeared to be dead.

Laboratory Results: Of the 30 oysters examined by PCR, 24 (80%) were confirmed positive for *Marteilia sydnei*. Confirmation of a successful DNA extraction from each oyster was done using a second PCR specific for *Saccostrea glomerata* (Sydney rock oyster) DNA. Positive and negative controls were included in each PCR run. Of the oysters positive on PCR, 11 (46%) were positive for *Marteilia sydnei* on cytological examination of digestive gland impression smears (stained with Diff Quik). All oysters positive on cytological examination were also positive on histological examination.

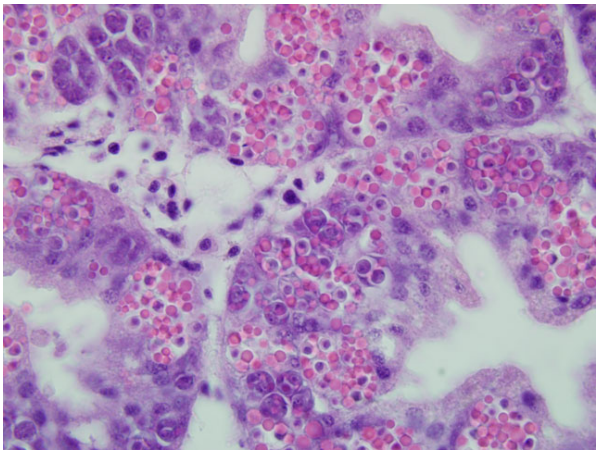
Histopathologic Description: Whole body: Multifocally there is marked disruption of digestive gland architecture with moderate inflammatory cell infiltration of the epithelium and myriad intracellular protozoa. Tubule epithelium is expanded by numerous multicellular protozoa consisting of large, 100-150 µm sporangiosorae containing 8-16 sporonts, each 10-15 µm, tear-shaped and 2-3 spherical, refractile eosinophilic spores. Occasional intraluminal sporangiosorae are noted. There is marked increase in granular enterocytes with diapedesis of haemocytes across tubule epithelium. Surrounding Leydig tissue is diffusely collapsed and infiltrated by low to moderate numbers of haemocytes. Underlying the gill and palp epithelium, moderate infiltrates of haemocytes are noted diffusely in the Leydig tissue.

Contributor's Morphologic Diagnosis: Digestive gland: Adenitis, proliferative, chronic, multifocal, severe, with haemocyte accumulation and myriad intracellular protozoa consistent with *Marteilia sydnei*; Sydney rock oyster (*Saccostrea glomerata*).

Contributor's Comment: Diseases caused by *Marteilia refingens* and *Marteilia sydnei* (QX disease) are referred to as Martiellosis. These organisms are of the phylum Paramyxea and are regarded as major concerns for mollusk aquaculture and are listed by the Office International des Epizooties (OIE 2003).

QX disease has been identified in Sydney rock oysters (*Saccostrea glomerata*) in coastal estuaries of southern Queensland and northern New South Wales. Infected oysters are typically in poor condition, with resorption of the gonad and pale digestive glands. Epizootics occur in summer and autumn and mortality can exceed

1-1. Digestive gland, Sydney rock oyster, (Saccostrea glomeratus). The glandular architecture is altered by epithelial hypertrophy and hyperplasia, with few inflammatory cells. Epithelial cells are filled with myriad intracytoplasmic protozoa. (HE 400X)



90%.[1] The marked increase in prevalence of QX disease and the devastating effect on the local industry has led to the development of a line of QX resistant oysters. Phagocytosis of *M. sydnei* by haemocytes is a key event in clearing the infection.[5] Interbreeding and investigation of the rare QX-infected surviving oyster demonstrated increased haemocyte-localized phenoloxidase concentrations.[7] While oysters selectively bred to be resistant to QX had increased concentrations of phenoloxidase, management of the disease continues to be problematic, in addition to the inadvertent breeding of oysters with reduced phenoloxidase concentrations.[3]

Gross lesions in QX affected oysters are distinctive, with shrinkage of the oyster, poor body condition and a pale, translucent appearance to the oyster and the digestive gland in particular.

The life cycle of *M. sydnei* is well characterized within the oyster and involves an intermediate host, believed to be a polychaete worm.[2] Initial entry of *M. sydnei* occurs at the gills and palps, with localization of initial infective stages in epithelia and systemic spread via the haemolymph and connective tissues to the epithelia of the digestive gland. While epithelial hyperplasia, hypertrophy and fusion of gill filaments are a feature of this stage of infection,[2] they were not noted in this case. As losses had been occurring for several weeks prior to submission, it is possible that systemic spread had occurred. Haemocyte accumulates were noted in the underlying Leydig tissue.

Presporulating stages (sporonts) are found in the digestive gland epithelium and occasionally in the surrounding connective tissues. Development of sporonts is characterized by the production of internal offspring following internal (cell-within-cell) cleavage, without the formation of spores (extrasporogenic proliferation). However, once established in the digestive gland, sporulating forms (sporangiosorae) are formed, containing 8-16 sporonts. Final cleaveage of sporonts results in the formation of 2-3 multinucleated spores delimited by a continuous wall. The subsequent release of spores into the lumen results in destruction of the host digestive gland epithelium. Death of the oyster infected with *M. sydnei* is believed to be due to blockage of the digestive tract with subsequent starvation of the oyster.[2] The unique feature of internal cleavage during sporulation differentiates *Marteilia* spp. from other protista.

The digestive gland is the primary focus of sporulation of *M. sydnei*. It is one of the principle sites of storage of metabolic reserves and of intracellular food digestion. It is a compound tubular organ with primary ducts leaving the stomach, secondary ducts and digestive tubules. Three populations of lining cells can be identified: digestive or secretory-absorptive cells, non-flagellated basiphil cells, and flagellated basiphil cells. Digestive cells are distinctive for their macrovesicles which contain various enzymes (acid phosphatase, non-specific esterases) and have a distinctive eosinophilic appearance in H&E.[4] In a healthy actively-feeding oyster, the lumina of the digestive gland have an "X" or "Y" appearance in cross section. Conversely, in unhealthy, stressed or non-feeding oysters, the digestive glands are dilated with a low cuboidal epithelium. Diapedesis of a small number of haemocytes is regarded as normal and represents movement of haemolymph across mucosal surfaces.[4]

AFIP Diagnosis: 1. Digestive gland: Adenitis, proliferative, diffuse, marked, with epithelial hypertrophy, mild haemocytic inflammation and myriad intracellular epithelial protozoa.
2. Gill: Branchitis, haemocytic, multifocal, mild with epithelial degeneration, necrosis, and sloughing.

Conference Comment: The moderator and participants discussed several considerations when approaching aquaculture disease from a diagnostic perspective. First and foremost, one must understand this is a population health issue which impacts the approach to the types and numbers of samples obtained. In general, one should collect fresh, frozen and fixed diagnostic samples to facilitate multiple diagnostic modalities. Aquaculture fixatives consist of Davidson's or saltwater-formalin to maintain osmotic balance.

As noted by the contributor, digestive cells have distinct eosinophilic macrovesicles. Participants were unsure of the exact nature of this unique feature. A brief discussion of the possibilities for a round

eosinophilic body included apoptotic bodies, granules/ vacuoles, viral inclusions and protein droplets. An example of protein droplets is the hyaline droplet nephropathy in rats with histiocytic sarcoma. The participants agreed that the feature is consistent with a vacuole.

In addition to the histologic lesions in the digestive gland, several conference participants observed multifocal haemocytic infiltrates in the gill along with degeneration, necrosis, and sloughing of the epithelial cells. The cause of the lesions in the gills is uncertain, but a few participants speculated on the possibility of opportunistic infection in the terminal stages of martiellosis, such as that caused by ciliated protozoa. Rare ciliated protozoa with a large basophilic macronucleus are observed in a few sections on the surface of the gill epithelium.

The contributor provides a detailed discussion of the epidemiology and pathology associated with martiellosis as well as the histologic features of the mollusk digestive tract. The following mollusk diseases also are listed as current OIE reportable entities: *Bonamia ostreae, B. exitosa, Marteilia refringens, Perkinsus marinus, P. olseni, Xenohaliotis californiensis*, and abalone herpes-like virus. Of these organisms, *B. ostreae, P. marinus*, and *X. californiensis* have affected mollusks native to the United States.[6]

Contributor: State Veterinary Laboratory, EMAI, Narellan, NSW, Australia
www.dpi.nsw.gov.au

References:

1. Adlard RD, Ernst I. Extended range of the oyster pathogen *Mautiella sydnei*. *Bull Eur Assoc Fish Pathol*. 1995;15(4):119.
2. Berthe FCJ, Le Roux F, Adlard RD, Figueras A. Marteiliosis in mollusks: a review. *Aquat Living Resour*. 2004;17:433-488.
3. Bezemer B, Butt D, Nell J, Adlard R, Raftos D. Breeding for QX disease resistance negatively selects one form of the defensive enzyme, phenoloxidase, in Sydney rock oysters. *Fish Shellfish Immunol*. 2006;20:627-636.
4. Kennedy VS, Newell RIE, Eble OF, eds. In: *The Eastern Oyster* Crassostrea virginica. College Park, MD: Maryland Sea Grant College; 1996:39-51.
5. Kuchel RP, Aladaileh S, Birch D, Vella N, Raftos DA. Phagocytosis of the protozoan parasite *Marteilia sydnei* by Sydney rock oyster (*Saccostrea glomerata*) hemocytes. *J Invert Pathol*. 2010;104(2):97-104.
6. Arzul I, Burreson EM, Friedman C. Diseases of mollusks, sections 2.4.2-2.4.7. In: *Manual of Diagnostic Tests for Aquatic Animals 2010*. OIE; http://www.oie.int/eng/normes/fmanual/A_summry.htm. Accessed online10 January 2011.
7. Peters R, Raftos DA. The role of phenoloxidase suppression in QX disease outbreaks among Sydney rock oysters (*Saccostrea glomerata*). *Aquaculture*. 2003;223:29-39.

CASE II: 06V3449 (AFIP 3170675).

Signalment: 2-year-old, mixed gender, greenlip abalone (*Haliotis laevigata*).

History: An abrupt onset of high mortality affecting 1 to 4-year-old juveniles and brood stock occurred on three abalone farms in southern Australia. Prior to the onset of these mortalities, abalone had been collected from the wild and there was transfer of stock between the farms. High mortality was observed in affected tanks with many tanks having mortalities of greater than 50%. Abalone died within 1-3 days after onset of clinical signs. Clinical signs included reduced pedal adhesion to the tank surface, loss of the righting reflex, and swollen mouths, sometimes prolapsed, with eversion of the radula.

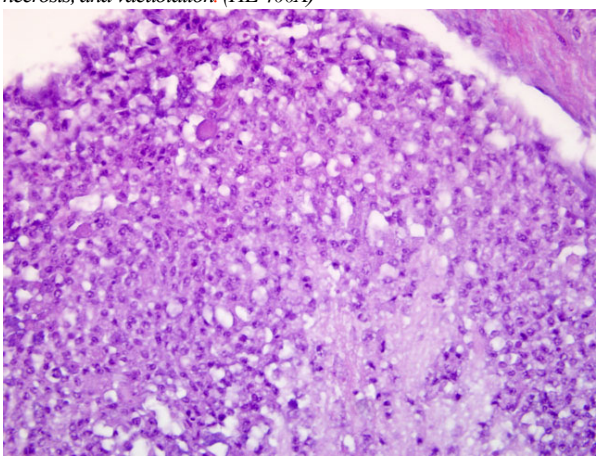
Laboratory Results: Hemolymph samples collected from the pedal sinus were plated onto Horse Blood Agar, TCBS, and McConkey's Agar and incubated at 20°C for 2 days. No significant pathogens were isolated.

Gross Pathology: Apart from the swollen mouths and prolapsed radula, no significant gross lesions were present.

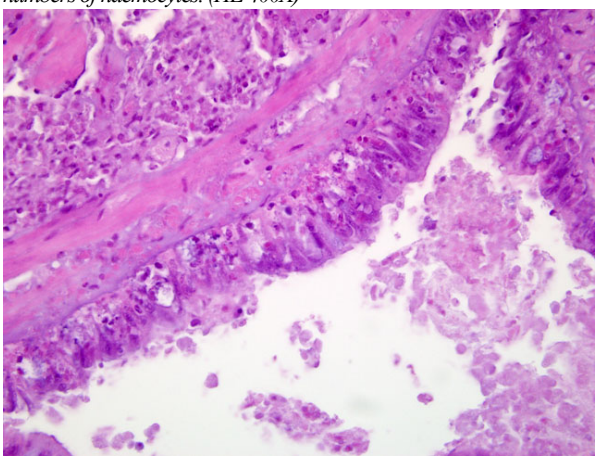
Histopathologic Description: The lesions are confined to nervous tissue and centered on the cerebral, pleuropedal and buccal ganglia, the cerebral commissure and the peripheral nerves arising from these structures. The lesions are an increased cellularity and individual cell necrosis in the affected nerves. Multifocally, the cell necrosis and edema cause a ragged and disheveled appearance in the neuropil. Occasional neurons have marginated chromatin and central pallor, but no Cowdry type A inclusions are present. The lesions are often strikingly well demarcated, with musculature directly adjacent to affected large nerves and ganglia having no lesions. Those abalone with swollen mouths have dilated sinuses and ruptured connective tissue leading to poorly delineated spaces, some of which are filled with hemolymph fluid and haemocytes.

Contributor's Morphologic Diagnosis: Severe multifocal necrotizing ganglioneuritis.

2-1. Cerebral commissure, abalone, (Haliotis laevigata). The neuropil is infiltrated and replaced by many haemocytes, with degeneration, necrosis, and vacuolation. (HE 400X)



2-2. Radula, abalone, (Haliotis laevigata). Multifocally there is degeneration and necrosis of the epithelium with infiltration of low numbers of haemocytes. (HE 400X)



Contributor's Comment: Abalone viral ganglioneuritis is a recently defined contagious viral disease of abalone caused by infection with abalone herpes-like virus (AbHV).[2] The disease was first reported in Taiwan[1] and more recently in Australia.[2] The relationship between the Australian viral isolate(s) and other herpes-like viral isolates has not, as yet, been elucidated, but it is suggested that this virus is the second member of the Malacoherpesviridae along with Ostreid Herpesvirus-1.[3]

The outbreaks occurred almost simultaneously in three farms following abalone movements from the wild and between farms. An opportunistic bacterial infection was quickly ruled out because several features differed from previous outbreaks of bacterial infection. These included the clinical signs, the pattern of spread of the outbreak on the initial farm, the very high rates of morbidity and mortality in some tanks during the first and subsequent weeks of the outbreak, and the links between movement of stock and subsequent disease outbreaks. The level of both wild broodstock collection and inter-farm exchange was greater this year than previous years due to efforts to increase and share genetic diversity in farms involved with an industry breeding program. Issues of translocation were considered following the national abalone disease survey.[3] Few significant translocation risks were identified in this process; those recognized, such as *Perkinsus olseni*, were taken into account and abalone were collected from areas outside their known distribution.

The lesions centered on neural tissue innervating the mouth parts of the abalone resulting in the clinical signs of swelling and prolapse of the mouth and eversion of the radula. The pleuropedal ganglion and pedal nerves were also commonly affected, leading to paralysis of the foot muscle and loss of adhesion to the tank surface.

The very high mortality, unusual clinical observations in moribund abalone, on-farm and between-farm pattern the outbreak, and the history of abalone movements linking farms were all highly suggestive of a new virulent infectious agent. As new animal and aquatic species become farmed commercially, health surveillance of these species will need to be developed and veterinarians and veterinary laboratories are key players.

AFIP Diagnosis: 1. Neural tissue, cerebral commissure, ganglia, and peripheral nerves: Ganglioneuritis, necrotizing, multifocally extensive, marked with haemocytic inflammation and rare intranuclear inclusion bodies.
2. Radula: Epithelial degeneration and necrosis, multifocal, moderate, with mild haemocytic inflammation.

Conference Comment: Species of abalone reportedly susceptible to herpesvirus infection include the greenlip abalone (*Haliotis laevigata*), the blacklip abalone (*H. rubra*) and *H. diversicolor supertexta* in Chinese Taipai. The initial outbreak in Australia occurred in the summer. Once established in wild abalone populations, morbidity and mortality can be seen throughout the year and appears not to be influenced by seasonality. The moderator commented that this particular entity is spreading and could reach the United States in the near future.[2] Diagnosis of abalone herpes-like virus can be accomplished by histopathology, *in-situ* hybridization, and electron microscopy where viral particles display ultrastructural characteristics of herpesrviridae.[2]

In addition to the histologic lesions in the cerebral commissure and ganglia, several conference participants observed multifocal epithelial degeneration and necrosis in the radula. The cause of the epithelial lesions in the radula is uncertain, but

most participants considered the change likely due to the sequela of herpesviral infection, and possibly an acute terminal lesion associated with paralysis of the mouth parts described clinically by the contributor.

Contributor: Gribbles Veterinary Pathology, 1868 Dandenong Rd Clayton, Melbourne, Victoria, Australia, 3168

References:
1. Chang P, Kuo S, Lai S, et al. Herpes-like virus infection causing mortality of cultured abalone *Haliotis diversicolor supertexta* in Taiwan. *Dis Aquat Org*. 2005;65:23-27.
2. Crane M, Corbeil S. Infection with abalone herpes-like virus. In: *Manual of Diagnostic Tests for Aquatic Animals 2010*. OIE; www.oie.int/eng/normes/fmanual/2.4.01_INF_ABALONE.pdf. Accessed online 10 January 2011.
3. Handlinger J, Bastianello S, Callinan R, et al. Abalone aquaculture subprogram: A national survey of diseases of commercially exploited abalone species to support trade and translocation issues and the development of health surveillance programs. Canberra, Australia: Fisheries Research and Development Corporation; FRDC Project No 2002/20:2006.
4. Hooper C, Hardy-Smith P, Handlinger J. Ganglioneuritis causing high mortalities in farmed Australian abalone (*Haliotis laevigata* and *Haliotis rubra*). *Aust Vet J*. 2007;85:188-193.

CASE III: WNo3/1247/22G (AFIP 2890835).

Signalment: Juvenile (3-4 month-old), male and female, giant tiger shrimp (*Penaus monodon*).

History: *Penaus monodon* ponds on farms in eastern Australia are typically 1 hectare in area and carry approximately 40 shrimp per square meter during the 5-6 month grow-out period. These moribund, lethargic shrimp were collected when excessive numbers (typically > 20 moribund or dead shrimp per day) were observed at pond edges during the second half of the grow-out period.

Gross Pathology: Affected shrimp were typically reddish in color, with mild to moderate epibiotic fouling and one or more partially amputated appendages.

Histopathologic Description: Whole body: In peripheral nerves, particularly antennal nerves, there is mild to severe, focal to diffuse degeneration and necrosis of axons and their sheaths, together with associated glial cell apoptosis. There is mild to severe, acute retinitis associated with degeneration and necrosis of retinular cells and their axons in the fasciculated zone of the photoreceptor region of the eye. In the lymphoid organ (located cranial to the hepatopancreas) there is moderate to marked increase in numbers of spheroids (aggregates of haemocytes). In the vas deferens of male shrimp, there is moderate to severe focal to locally extensive epithelial necrosis.

In the putative glial cells in the antennal nerve and in the fasciculated zone of the photoreceptor region of the eye, moderate to large numbers of intracytoplasmic rod-shaped, helical nucleocapsids and enveloped virions, morphologically consistent with a yellow head-like virus (Order Nidovirales), were demonstrated by transmission electron microscopy.

By immunohistochemical examination using monoclonal antibodies against yellow head-related viruses, tissues containing lesions (but not histologically normal tissues in the affected shrimp) consistently stained positively.

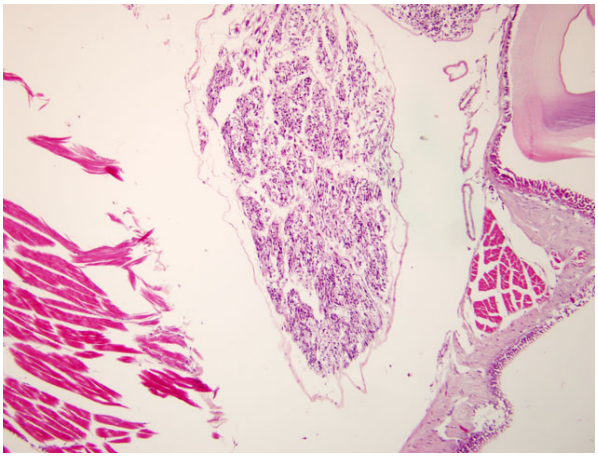
Contributor's Morphologic Diagnosis: 1. Peripheral neuropathy, necrotizing, acute, segmental, severe.
2. Retinopathy, necrotizing, acute, multifocal, severe (peripheral neuropathy and retinopathy disease).

Contributor's Comment: Since 1994 significant mortalities during the second half of the grow-out period on eastern Australian *P. monodon* farms have often been attributed by farmers to a condition designated as "midcrop mortality syndrome" (MCMS). It is likely that "peripheral neuropathy and retinopathy" (PNR), the emerging disease diagnosed in the current cases, is a component of disease within the MCMS complex.[1,2] PNR has been recorded only in farmed *P. monodon* from eastern Australia.

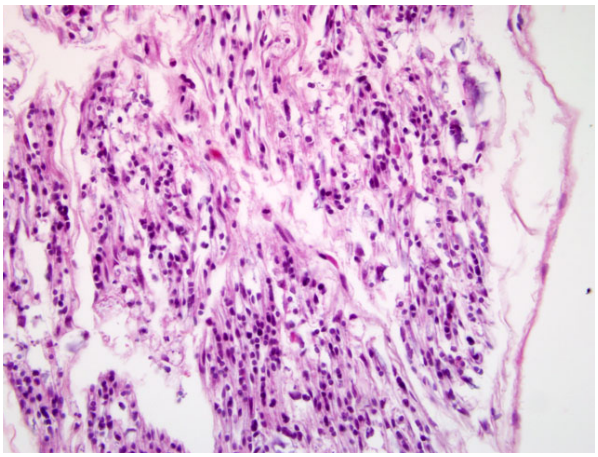
Outbreak studies strongly suggest that a virus morphologically and immunologically similar to viruses in the yellow head group is the causative infectious agent of PNR. Gill-associated virus (GAV) is the only such virus so far described from eastern Australia, where it is widely endemic in wild and farmed *P. monodon* populations. Evidence suggests GAV is transmitted both vertically in shrimp hatcheries and horizontally via cannibalism in ponds.[2,3]

Production losses are usually minimal if ponds experiencing PNR outbreaks are harvested within three weeks of outbreak recognition. Severe losses (up to 50% mortality) may occur when harvest is delayed for longer periods.[2]

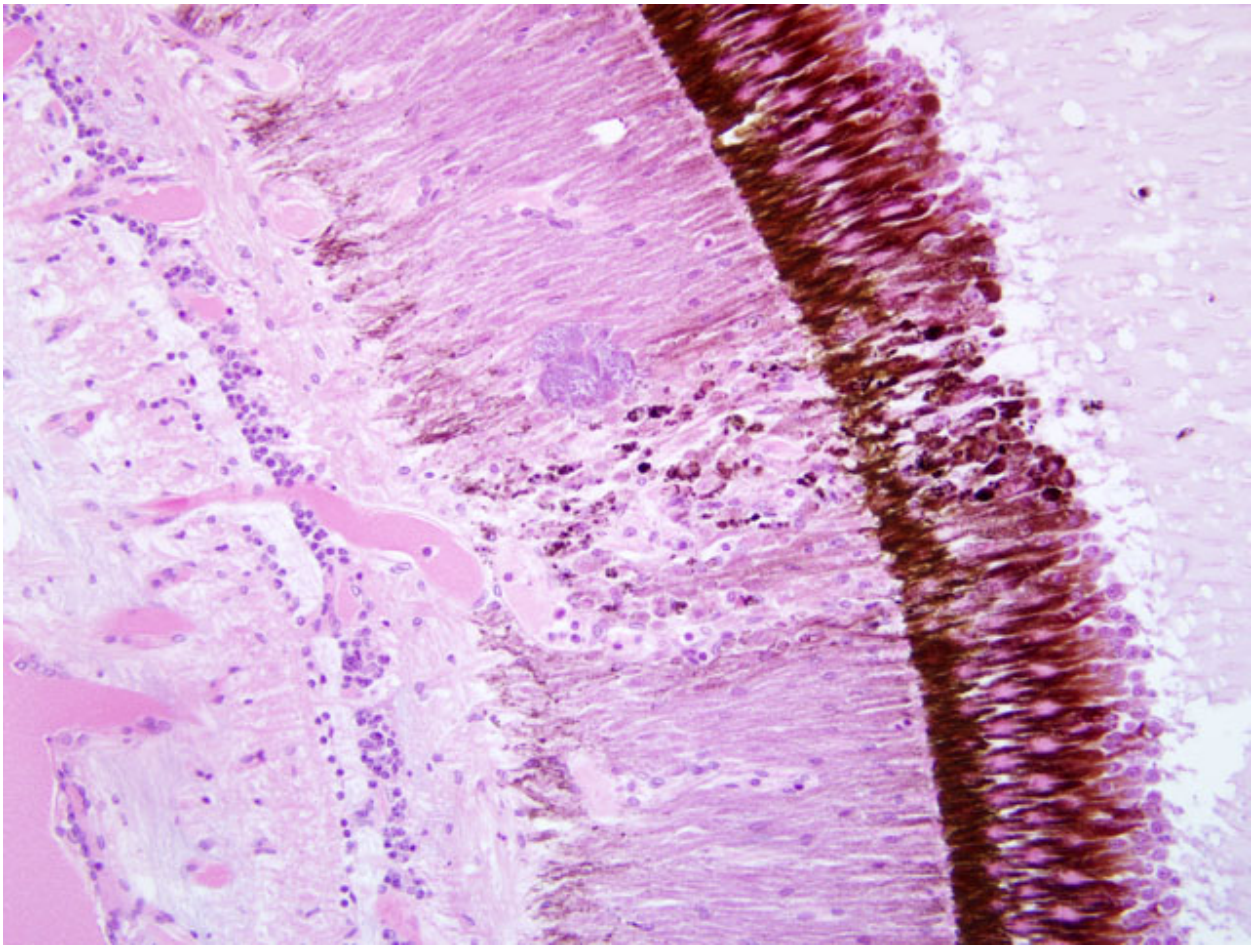
AFIP Diagnosis: 1. Peripheral nerve: Radiculoneuritis, haemocytic and necrotizing, multifocal, moderate.
2. Eye, retina: Retinular cell degeneration and necrosis, multifocal, moderate with axonal degeneration.



3-1. Peripheral nerve, giant tiger shrimp (Peneus monodon). Peripheral nerves are infiltrated and expanded by moderate numbers of haemocytes. (HE 100X)



3-2. Peripheral nerve, giant tiger shrimp (Peneus monodon). Peripheral nerves are infiltrated by moderate numbers of haemocytes, and there is associated axonal degeneration, vacuolation, and loss. (HE 400X)



3-3. Eye, retina, giant tiger shrimp (Peneus monodon). Retinular cells are degenerate and necrotic, with infiltration by low numbers of haemocytes. (400X)

Conference Comment: Gill-associated virus (GAV), the type species for the genus *Okavirus*, is one of six genotypes; it is designated as genotype 2 and, along with genotypes 3-6, commonly infects *Penaeus monodon* without causing disease. Yellow head virus (YHV) is designated as genotype 1 and tends to be more virulent than the other five genotypes. Viruses in this group are enveloped with positive-sense, single-stranded RNA. Ultrastructurally, the *Okaviruses* are rod-shaped, enveloped with peplomeres, contain a helical nucleocapsid, and measure 40-60 nm x 150-200 nm. The virus targets ectodermal and mesodermal tissues, notably lymphoid organs, hemocytes, hematopoietic tissues, gill lamellae and nervous tissue. Initial pond infection with YHV and GAV can produce up to 100% and 80% mortality, respectively. Outbreaks are often associated with physiologic stress such as pH or oxygen saturation change.[3]

Diagnosis is suspected by clinicopathologic presentation and confirmed by a variety of ancillary laboratory methods. Affected animals cease feeding and congregate at the pond edges and surface. Macroscopically, animals have a bleached appearance with yellowing of the cephalothorax and pink to yellow discoloration of the gills. Histologically, detection of deeply basophilic, round, intracytoplasmic viral inclusions, particularly in the lymphoid organ, stomach subcuticulum, and gills is often diagnostic. Electron microscopy, wet mounts, fixed hemolymph smears, rt-PCR, *in-situ* hybridization and bioassays are other confirmatory diagnostic tools.[3]

Contributor: NSW Agriculture, Wollongbar Agricultural Institute, Regional Veterinary Laboratory, Wollongbar, NSW, Australia.

References:

1. Callinan RB, Jiang L, Smith PT, Soowannayan C. Fatal, virus-associated peripheral neuropathy and retinopathy in farmed *Penaeus monodon* in eastern Australia: I. Pathology. *Dis Aquat Org*. 2003;53:181-193.
2. Callinan RB, Jiang L. Fatal, virus-associated peripheral neuropathy and retinopathy in farmed *Penaeus monodon* in eastern Australia: II. Outbreak descriptions. *Dis Aquat Org*. 2003;53:195-202.
3. Walker P. Yellow Head Disease. In: *Manual of Diagnostic Tests for Aquatic Animals 2010*. OIE; http://www.oie.int/eng/normes/fmanual/2.2.07_YHD.pdf. Accessed online 10 January 2011.
4. Walker PJ, Cowley JA, Spann K, Hodgson RA, Hall M, Withyachumnarnkul B. Yellow head complex viruses: Transmission cycles and topographical distribution in the Asia-Pacific region. In: Browdy CL, Jory DE, eds. *The New Wave*. Proceedings of the Special Session on Sustainable Shrimp Culture, Aquaculture 2001. The World Aquaculture Society, Baton Rouge, LA, USA.

CASE IV: 07F37 (AFIP 3065923).

Signalment: Adult, red swamp crayfish (*Procambarus clarkii*).

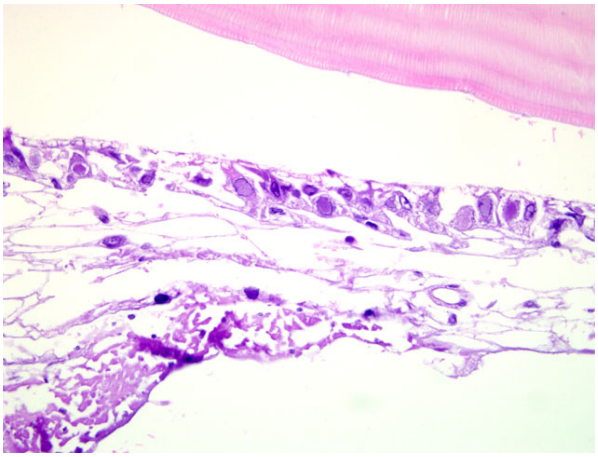
History: This spring a crayfish pond in southern Louisiana has been experiencing high mortality, especially evident in the larger specimens. Affected crayfish are lethargic.

Laboratory Results: In situ hybridization for white spot syndrome virus (WSSV), DNA on embedded tissue sections showed positive staining in affected nuclei. PCR testing (NVSL in Ames, IA) for WSSV in tissues was positive.

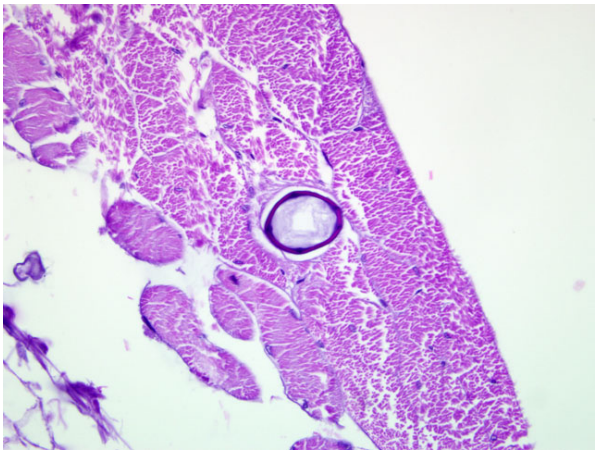
Gross Pathology: In some cases, crayfish exhibit marked gill fouling (accumulation of pond detritus on the gills and exoskeleton).

Histopathologic Description: Sections are composed of a transverse abdominal cross section (some slides contain gill tissue as well). There are large numbers of intranuclear inclusions in the cuticular epithelium, hindgut mucosa, and gill epithelium. Within the hindgut, luminal epithelium contains many inclusions with lesser numbers in the acinar gland epithelium and connective tissues. Occasional inclusions are present in the glial cells of the ventral nerve cord. Within the gill, many flange cells contain inclusions. Inclusions are eosinophilic to basophilic and occasionally have a clear halo (Cowdry type A). Affected cells have enlarged nuclei with marginated chromatin. Multifocally, along the abdominal dorsum, there is cuticular epithelial loss and necrosis with cuticular separation and suffusion of proteinaceous fluid (hemolymph) into the intervening space. Multifocally, muscle tissues contain 100-250 micron long by 40-60 micron wide, sagittally acicular to ovoid, transversely polyhedral (4-6 sided) organisms with eosinophilic, birefringent, thick shell plates and a basophilic medial shell membrane (sporocyst). In cases where sporocyst internal tissues are preserved, there is a fine, lightly eosinophilic inner membrane encasing one to several, 10-15 micron wide, lightly eosinophilic amoeboid bodies (spores). Multifocally, myocyte bundles exhibit fine basophilic granularity (mineralization) with varying degrees of sarcoplasmal degeneration and necrosis.

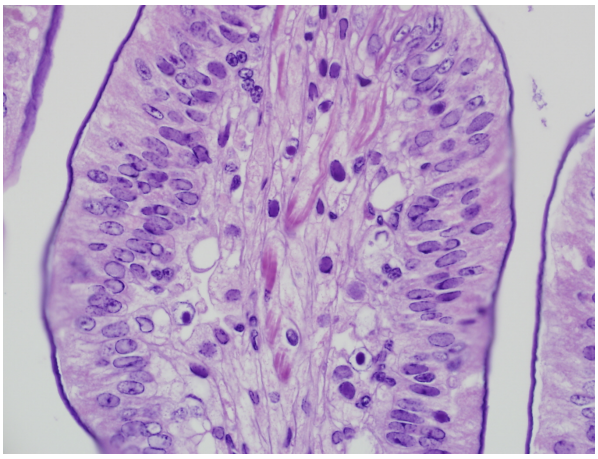
Contributor's Morphologic Diagnosis: Multiple eosinophilic to basophilic intranuclear inclusions and karyomegaly in ectodermal and mesodermal tissues; moderate, multifocal, cuticular epithelial necrosis with



4-1. Subcuticle, crayfish, (Procambarus clarkia). Intranuclear viral inclusions are present in many degenerate and necrotic subcuticular epithelial cells. (HE 400X)



4-2. Skeletal muscle, crayfish, (Procambarus clarkia). Parasitic sporocysts with an eosinophilic, birefringent, thick shell plate and a basophilic medial shell membrane are present within skeletal muscle. (HE 400X)



4-3. Hindgut, crayfish, (Procambarus clarkia). Mucosal epithelial cells contain basophilic intranuclear viral inclusion bodies. Photograph courtesy of Louisiana State University School of Veterinary Medicine, Department of Pathobiological Sciences, http://www.vetmed.lsu.edu/pbs/

cuticle separation; multiple intramuscular parasitic sporocysts (*Psorospermium* sp.); mild, multifocal myocyte necrosis (white spot syndrome virus, p sorospermiasis).

Contributor's Comment: White spot disease (WSD) is caused by the etiologic agent white spot syndrome virus (WSSV). The disease is so named due to the development of white, circular to coalescing spots in the cuticles of afflicted penaeid shrimp which are formed by the deposition of $CaCO_3$, cell debris, chitin, protein, and tegumental gland products as a result of cuticular pore canal destruction.[12,14] It is, unfortunately, a nonspecific alias since the formation of cuticular white spots may also be caused by bacterial infection or elevated water pH.[12] Since the first reported occurrence in 1992, WSD has had a profoundly detrimental impact on the cultured shrimp industries in Asia, Latin America, and the United States of America.[3,5] The beginning of an outbreak is heralded by anorexia; then the appearance within days of moribund shrimp at the water surface; and the development of white spots in some individuals.[1] Outbreaks often have high daily mortality rates with total mortality reaching 100% within 3 to 10 days.[6] All decapod crustaceans are considered susceptible, inclusive of marine, brackish, and freshwater species, as well as all life stages from egg to brood stock. Transmission may occur vertically (trans-ovum) or horizontally through ingestion of infected tissue and waterborne routes. Morbidity and mortality are highly variable between species.[8] In crayfish, WSSV infection clinically causes lethargy and grossly produces mottling or slight discoloration of the cuticle. Mortality rates both experimentally and in outbreaks have been high.[9,11]

The white spot syndrome virus is a double stranded, non-occluded, enveloped, DNA virus currently in the family *Nimaviridae*, genus *Whispovirus*, but had historically been assigned to the *Baculoviridae* family.[1] Virions are large and elliptical to rod shaped, measuring 70-150 nm by 250-380 nm.[6] Infected cells exhibit nuclear hypertrophy, chromatin margination, and eosinophilic to basophilic inclusions. The central aspect of inclusions is composed of unformed virogenic material admixed with formed virions, while mature virions tend to aggregate at nuclear margins in ordered arrays. Progressive infection leads to nuclear dissociation and cell death.[3,14] The WSSV infects tissues of ectodermal and mesodermal origin, namely the foregut, hindgut, gills, antennal gland, integument, gonads, muscle, heart, hemocytes, nervous tissue, and hematopoietic tissue. Considerable debate still exists, however, as to the site of entry, primary replication, and method of spread of WSSV *in vivo*.[5]

Histologically, prominent eosinophilic to basophilic intranuclear inclusions are present most commonly in the cuticular epithelium, connective tissues, gills, foregut, and hindgut.[6,7] The presence of Cowdry type A inclusions is considered indicative of early inclusion body development. Likewise, the progression of inclusions from eosinophilia to basophilia is considered a hallmark of advanced infection. Furthermore, these features are used by some to distinguish WSSV from other viral infections.[6,15] Caution is warranted, however, as assessment of inclusions is often based on sections stained by a specific published protocol by T. Bell and Don Lightner. Transmission electron microscopy (TEM), in-situ hybridization, or various PCR methods are necessary for confirmation.[8]

Psorospermium belongs to the class *Mesomycetozoea*, a protistan clade located near the animal-fungal taxonomic divergence.[10] Very little is known about the life cycles and feeding habits of the members of this class. The pathogenicity of this organism in crayfish is uncertain due to the variance in reported host reaction.[4] Originally described in 1857, "the enigmatic parasite of freshwater crayfish" was finally determined to be part of the DRIP clade (*Dermocystidium*, rosette agent, *Ichthyophonus*, and *Psorospermium*) in 1997, which has subsequently been placed in *Mesomycetozoea*. *Psorospermium haeckeli* is the only confirmed species, although others have been described but remain to be accepted.[10] *Psorospermium* spp. has been documented in 15 crayfish species in Europe, North America, and Australia.[13] In astacid crayfish this organism is found most often in connective and epidermal tissues under the carapace. In cambarid crayfish (*P. clarkii*, among others), it is most often present in abdominal muscles.[4]

AFIP Diagnosis: 1. Cuticle and subcuticle: Epithelial degeneration and necrosis, multifocally extensive, moderate with subcuticular separation, edema, karyomegaly and intranuclear inclusion bodies.
2. Skeletal muscle: Intramyocytic basophilic cysts.
3. Alimentary tract, epithelium and subepithelial connective tissue: Karyomegaly, with amphophilic intranuclear inclusion bodies.

Conference Comment: Scientific knowledge of this economically important infectious disease is relatively sparse. As the contributor notes, there are still several unknown factors in the epidemiology and pathogenesis of white spot disease. For instance, the virus can be transmitted both horizontally and vertically; infection can also occur between outwardly healthy, apparently disease-free animals and susceptible animals. The triggers for individual disease development are unknown, with some infections being subclinical while outbreaks have been associated with changes in environmental factors. Lifelong infection of carriers is also common.[2]

In addition to the diagnostic modalities listed by the contributor, there are several, often less labor-intensive, diagnostic techniques available. Clinically, hemolymph from infected shrimp often has delayed or absent clotting reaction. Stained or unstained wet mounts of the gills and cuticular epithelium may readily demonstrate karyomegaly. Dark-field microscopy of unstained hemolymph will show viral aggregates as 0.5 μm reflective spots. White spot syndrome virus has a unique ultrastructural appearance; it is described as bacilliform, being ~300 nm in length by ~150 nm in width. Negatively stained electron micrographic studies may reveal the presence of a tail-like appendage extending from one end of the virion.[2]

Contributor: Louisiana State University School of Veterinary Medicine, Department of Pathobiological Sciences, Skip Bertman Drive, LSU, Baton Rouge LA, 70803
http://www.vetmed.lsu.edu/pbs/

References:
1. Anonymous. White spot disease. In: *OIE Manual of Diagnostic Tests for Aquatic Animals 2003*. 4th ed. Paris, France: OIE; 2003:285-297.
2. Chu-Fang Lo G. White spot disease. In: *Manual of Diagnostic Tests for Aquatic Animals 2010*. OIE; http://www.oie.int/eng/normes/fmanual/2.2.05_WSD.pdf. Accessed online 11 January 2011.
3. Durand S, Lightner DV, Redman RM, Bonami JR. Ultrastructure and morphogenesis of white spot syndrome baculovirus (WSBV). *Dis Aquat Org*. 1997;29:205-211.
4. Edgerton BF, Evans LH, Stephens FJ, Overstreet RM. Review article: Synopsis of freshwater crayfish diseases and commensal organisms. *Aquaculture*. 2002;206:57-135.
5. Escobedo-Bonilla CM, Wille M, Alday Sanz V, Sorgeloos P, Pensaert MB, Nauwynck HJ. Pathogenesis of a Thai strain of white spot syndrome virus (WSSV) in juvenile, specific pathogen-free *Litopenaeus vannamei*. *Dis Aquat Org*. 2007;74:85-94.
6. Lightner DV. *A Handbook of Pathology and Diagnostic Procedures for Diseases of Penaeid Shrimp*. Baton Rouge, LA: World Aquaculture Society; 1996.
7. Lo CF, Ho CH, Chen CH, et al. Detection and tissue tropism of white spot syndrome baculovirus (WSBV) in captured brooders of *Penaeus monodon* with a special emphasis on reproductive organs. *Dis Aquat Org*. 199730:53-72.
8. Lo G. Chapter 2.3.2 White spot disease. In: Commission OAAHS, eds. *OIE Manual of Diagnostic*

Tests for Aquatic Animals (online). Paris, France: OIE; 2006.
9. Maeda M, Itami T, Mizuki E, et al. Red swamp crawfish (*Procambarus clarkii*): An alternative experimental host in the study of white spot syndrome virus. *Acta Virol*. 2000;44:371-374.
10. Mendoza L, Taylor JW, Ajello L. The class Mesomycetozoea: A heterogeneous group of microorganisms at the animal-fungal boundary. *Annu Rev Microbiol*. 2002;56:315-344.
11. Richman LK, Montali RJ, Nichols DK, Lightner DV. A newly recognized fatal baculovirus infection in freshwater crayfish. In: *Proceedings of the American Association of Zoo Veterinarians*. 1997;262-264.
12. Sahoo AK, Patil P, Shankar KM. White spots? A loaded question for shrimp farmers. *Curr Sci*. 2005;88:1914-1917.
13. Vogt G, Rug M. Life stages and tentative life cycle of *Psorospermium haeckeli*, a species of the novel DRIPs clade from the animal-fungal dichotomy. *J Exp Zool*. 1999;283:31-42.
14. Wang YG, Hassan MD, Shariff M, Zamri SM, Chen X. Histopathology and cytopathology of white spot syndrome virus (WSSV) in cultured *Penaeus monodon* from peninsular Malaysia with emphasis on pathogenesis and the mechanism of white spot formation. *Dis Aquat Org*. 1999;39:1-11.
15. Wongteerasupaya C, Vickers JE, Sriurairatana S, et al. A non-occluded, systemic baculovirus that occurs in cells of ectodermal and mesodermal origin and causes high mortality in the black tiger prawn *Penaeus monodon*. *Dis Aquat Org*. 1995;21:69-77.