Pathology and Parasitology Special Session    October 22

Hosted by Asian Society of Wildlife Pathology and Parasitology

Asian Zoo/ Wildlife Histopathology and Parasitology Conference,  2011

The goal of slide conference for Asian Wildlife Pathology and Parasitology:

To promote slide-exchanging and joint ownership of zoo and wildlife cases among Asian pathologists and parasitologists who are interested in zoo and wild animals, we are planning to have a session of slide conference and case presentation in this 3rd International Workshop of Asian Society of Zoo and Wildlife Medicine in Kathomandu. This idea originated from that successful slide conference organized by National Taiwan University at the ASVP/ASZWM workshop in Taipei in August in 2007 (proposed by professor, Dr. V.F. Pang). We think, we are as pathologists and parasitologists need to have as much as opportunity to meet and discuss about various interesting cases of zoo and wild animals include various Asian mammals, reptiles, amphibians and fishes. Like previous slide conference in ASZWM meeting in Bogor (2008) and Seoul (2009) and Kuala Lumpur (2010), all conference participants were able to take a look some of cases at Web at the National Taiwan University.

Thank you.

Organizers: CR-Jeng (National Taiwan University), Tokuma Yanai (Gifu University and, S.H. Vincent Hsiao (Illnoi State University)

ASWPP is a friendly society of ASZWM.

Asian Society of Wildlife Pathology and Parasitology (ASWPP) was founded in 2006 as sister society of Asian Society of Zoo and Wildlife Medicine (ASZWM). It’s major purpose is as follows:

1. To hold slide conference at annual meeting (or workshop) of ASZWM
2. To make networking for pathologists who are interested in zoo and wildlife pathology and parasitology in Asia
3. To support young people who are interested in zoo and wildlife pathology and parasitology
In case of requiring helps on glass slide preparation, a formalin-fixed and paraffin-embedded tissue block large enough for making more than 20 tissue sections should be sent to Dr. CR-Jeng in National Taiwan University.

Microscopic slides or paraffin-embedded tissue blocks along with a brief case signalment should be submitted to Dr. CR Jeng Veterinary Pathology laboratories, Department of Veterinary Medicine, National Taiwan University, No.1, Section 4, Roosevelt Road, Taipei 106, Taiwan.

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Chairpersons: Dr. CR Jen (NTU) and Dr. Wijit Banlunara

Dr. CR Jen DVM, PhD, Professor of Veterinary Pathology, Veterinary College, National Taiwan University Email: crjeng@ntu.edu.tw
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Memo:
Case 1

Contributor
Chang tsung chou1 (張聰洲)、Wong sing ming1(翁興民)、Bong yih tyng1(王逸葶)、Wang jiang ping2(王建平)
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CASE HISTORY

Signalment :
Melon-headed whale, male, immature

Clinical History :
The patient was stranded and rescued for seventeen days with clinical signs of weakness, body wasting and poor appetite.

Gross lesions:
The gastric lesions were characterized mainly by the presence of ulceration and inflammation of the patient's fundic stomach with parasitism by nematodes, e.g. Anisakis sp. The small, multiple ulcers are typically shallow and have the anterior end of the worm embedded in the ulcerous regions; there were numerous adult and larval Anisakine parasites free within the lumen. The gastric mucosa presented edema and hyperemia.

CASE RESULT

Histopathological finding:
The histopathological characteristics are multiple ulceration with chronic granulomatous reaction. The chronic lymphoplasmocytic gastritis was observed eosinophilic and granulomatous inflammation with giant cells, dystrophic calcification, hemosiderosis, fibrosis and areas of necrosis were associated with location of degenerative parasites within the gastric mucosa.

Electron Microscopic Finding:
The worm characterized by SEM: cuticle has fine striations; three bilobed lips are presented one dorsal and two ventrolateral; a boring tooth is present; and excretory pore lies between the ventrolateral lips.

Morphologic diagnosis:
Gastric Granuloma; Anisakiasis
Discussion:

Anisakiasis is a human disease caused by the accidental ingestion of larval nematodes belonging to the family Anisakidae. There are two forms of anisakiasis: noninvasive and invasive. Invasive anisakiasis occurs when larval attach to embed in or penetrate host tissue. Anisakis spp. are most often implicated in this type of anisakiasis. They have been found in the mucosa or submucosa of the stomach or intestine and have migrated to other tissues such as the omentum, pancreas, liver and probably the lung (Kobayashi, 1985; Yokogawa, 1967). Symptoms of acute gastric aniskiasis include sudden epigastric pain, nausea, vomiting, diarrhea. Many acute cases are misdiagnosed as stomach ulcer, stomach cancer, and ileitis and the infection become chronic. Recently, diagnosis of anisakiasis has been successfully done by endoscopy, radiologic films, immunodiagnostic assays and histopathology.

The cetacean stomach is a diverticulated composite stomach, consisting of regions of stratified squamous epithelium, fundic mucosa, and pyloric mucosa. Ulceration and inflammation of the stomach can be attributed to parasitic and non parasitic. Parasites cause ulceration of the gastric mucosa and submucosa were predisposed by larvae penetrating the mucosa. Non parasitic ulceration can be attributed to histamine toxicosis.

As a result of Anisakiasis study in Japan, the histopathologic lesions have been classified into five types (i) the early stage: proliferation of neutrophils with few eosinophils and giant cells, little fibrinous exudation. (ii) first week: showed edematous thickening of submucosa with massive eosinophils. (iii) in chronic gastritis (< 6 months): abscess with eosinophilic ulceration may be seen. (iv) in gastric anisakiasis of > 6 months: granulomatous change with eosinophils might be found. (v) 6 months to years: the granulomatous inflammation with giant cells, lymphocytes, plasma cells and degenerated larvae are presented in the center of the lesions. The histopathological lesions of this patient is same type (v)

References:
Case 2
Shu-Chia Hu¹ DVM, MS; Victor Fei Pang¹ DVM, Ph D; Tzu-Ming Huang² DVM, Ph D; An Hsing Lee³
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CASE HISTORY:

Slide Number:
NTU2010-494

Signalment:
A 3-day-old male Mongolian wild horse (Equus przewalskii)

Clinical history
The animal had difficult to suck the milk after born.  The animal was weak and was not response to the
intensive medical care.

Gross findings
The nutritional condition and the post mortem condition of the animal were fair. No abnormality was noted
externally. The texture of the lung was elastic and un-collapsed. There was multifocal hemorrhage lesions
located at the leaflet of the mitral valves. Both sides of the kidneys were dark red in color with multiple
white-yellow or caseous-white foci, measured 0.1mm in diameter, present in the cut surface and in the
parenchyma. Similar lesions were extended into the liver. The mucosa of stomach was reddish, and there
were five segments of intussusceptions in jejunum with bloody contents. The amounts of synovial fluids
were increased and became yellowish in color, and most joints had the similar condition. Engorgement of
blood vessels was noted in cerebrum, especially in left cerebrum.

Laboratory Examinations
Actinobacillus equuli subsp. haemolytica are isolated from kidney, and Streptococcus equi
subsp. zooepidemicus are isolated from the synovial fluid swab of left hind limb.

CASE RESULT:

Histopathological Findings:
Multifocal to coalescing lesions are randomly distributed in the cortex and medulla of
Kidney, which consist of mononuclear cells, neutrophils, and bacterial colonies with
hemorrhage. The outline of glomerule are often lining with bacterial colonies and are
associated with the lesion aforementioned. The lesions extend from the glomerule to the interstitial tissue,
and the normal renal architecture is destroyed. In the liver, the lesions are similar as seen in the kidneys
which are characterized by large amounts of inflammatory cells aggregated with bacterial colonies in the
parenchyma. In H&E and B&B stain, the bacteria are Gram negative coccobacillus with 0.5 to 1 um in
Large areas of hemorrhage with scant macrophages infiltration are noted in the adipose tissues of heart, and one necrotic lesion with aggregation of inflammatory cells, bacterial clumps, and hemorrhage in the valves. The alveolar spaces are clear, but some parts of alveolar septa become thicker and contain inflammatory cells aggregation, most are lymphocytes with plasma cells and macrophages. The mucosa of small intestine, which intussusceptions was noted grossly, is necrosis with villi detachment, and the mucosa, submucosa, muscularis layer, and mesentery are congestion. The amounts of lymphocytes are less comparatively in some parts of lymph node.

In the blood vessel of umbilicus, large amount of neutrophils and mononuclear cells with bacterial colonies are noted.

**Diagnosis:**
1. Embolic nephritis, severe, multifocal to coalescing, subacute, purulent, with intralesional Gram negative coccobacillus, compatible with *Actinobacillus equuli* subsp. haemolytica kidney.
2. Hepatitis, mild, multifocal to coalescing, subacute, purulent, with intralesional Gram negative coccobacillus, liver.
4. Valvulitis, severe, locally, subacute, purulent and hemorrhagic, with bacterial clumps, Heart.
5. Enteritis, severe, locally extensive, acute, necrotizing, with congestion, compatible with intussusceptions, intestine.
5. Omphalitis, severe, locally, acute, with bacterial clumps, umbilicus
6. Lymphocyte depletion, mild, lymph node

**Discussion:**
The Przewalski’s horse (*Equus caballus przewalskii*) was extinct in the wild by the mid 1960s. The species has survived because of captive breeding only. The Takhin Tal reintroduction project is run by the International Takhi Group; it is one of two projects reintroducing horses to the wild in Mongolia.

From the literature review, disease information in this species is extremely rare and only two papers were found in the pubmed. One paper reported myelopathy and vitamin E deficiency in six Mongolian wild horses in Bronx Zoo. The other one reported “Pathologic findings in reintroduced Przewalski's horses (Equus caballus przewalskii) in southwestern Mongolia” . Equine piroplasmosis, a tick-transmitted disease caused by Babesia caballi or Theileria equi, is endemic in Takhin Tal and was identified as the cause of death of four stallions and one stillborn foal. Another notable disease in this study is the lesions compatible with strangles. Other occasional causes of death in this field study including trauma, exhaustion, wasting, urolithiasis, pneumonia, abortion, and stillbirth.

The Przewalski's horses have been kept in Taipei Zoo for more than three decades and there are usually maintained around 10 animals in this exhibition. The septicemic case of neonatal foal in this case may
resemble the cases often found in the domesticated foal. Septicemia is the major cause of death in foal of age less than 7 days, and *Actinobacillus equuli*, which isolated from the animal with the support by Dr. Huang of NIVR, is reported cause of an acute, highly fatal septicemia of new born foals, and is the 2nd most common isolated organism in the literatures. *Actinobacillus* sp, is reported to affect foals within a few hours of birth and up to 3 days of age. The pathogen may be acquired in utero, during parturition or shortly after birth; it is probably an umbilical infection. Death may occur due to fulminating septicemia. In this case, due to the animal were unable to suck the colostrum, early infection causes weakness of the animal or poor passive immunity, may both be the factors of severe bacterial infections in this case.

In addition to the isolation of *A. equuli*, *Streptococcus equi* subsp. *zooepidemicus* is also isolated, which may suggest the animal was suffered from mixed infections. Foals with bacteremia may have the history of colic (13% in research), and the intussusceptions found in this case may associate with this syndrome.

**References:**

Case 3

Tokuma Yanai(1), DVM, PhD; Walid Abdo(1), DVM; Hiroki Sakai(1), DVM, PhD; Hideto Fukushi(2), DVM, PhD; Hideko Okuda(2), DVM; Yuka Kakizoe(3), DVM; Masahito Ryono(3), DVM, PhD; Rui Kano(4), DVM, PhD; Tomoyuki Shibahara(5), DVM, PhD

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CASE HISTORY:

Signalment:
An adult female killer whale (Orcinus orca), 28 years old

Clinical history
The presented case had been moved to Port Nagoya Public Aquarium at the beginning of June in 2010 from another aquarium, where the whole had been maintained for over 20 years. The animal exhibited gradual loss of body weight over several months after its arrival. Signs of anorexia and gastrointestinal disorders first appeared at the beginning of October 2010. The animal developed severe anorexia in the middle of December 2010 and died on January 14, 2011.

Blood examination done on October 5, 2010 revealed an elevated total white blood cell count (WBC) (12,500/μl), fibrinogen level (344 mg/dl) and decreased alkaline phosphatase (ALP) (154 IU/l). Follow-up blood tests done 3 and 10 days later revealed the same results, with a decrease in the RBC count. Blood samples obtained on January 5, 2011 showed marked elevation of the WBC (21,250/μl), fibrinogen level (711 mg/dl), and aspartate aminotransferase (AST) (44 IU/l) and a decreased RBC count (306 x 10^6/μl) and Hb level (9.5 g/dl). In addition, the ALP value demonstrated a continuing decrease (93 IU/l). Blood analysis 7 days later (January 12) showed anemia, a sudden decrease in WBC (7,100/μl) and ALP (82.1 IU/l) and an increase in AST (109 IU/l), ALT (26 IU/l), along with continuous elevation of fibrinogen (834 mg/dl) and LDH (945 IU/l).

Gross findings:
At necropsy, the body weight was 2,450 kg, and the body measured 589 cm in length. The animal was poorly nourished. One of the most striking gross findings was the marked enlargement of the stomachs; three stomachs contained a large number of palpable stones measuring 1 to 15 cm in diameter. The wall of the first stomach was markedly thinned, and contained 474 various-sized stones, with a total weight of 69.2 kg. The second stomach had 16 stones weighing 11.5 kg in all. The third stomach contained one stone weighing 0.7 kg (120 x 80 x 60 mm). The stones ranged in size from a few cm to 17 cm in diameter, and in shape from round to oval, with smooth surfaces. Multiple petechial hemorrhages with severe erosions were observed in the gastric mucosa of the second and third stomachs. The thoracic lymph nodes were markedly enlarged and edematous. Both lungs were enlarged and were firm in texture, with numerous nodules, especially in the upper half of the lungs. On cut surfaces, there were severe diffuse consolidations, along with multi-focal liquefactions, as well as cavitations measuring 3 to 5 cm in size in the lungs. The cavities had coalesced to each other to form larger cavities, and were usually filled with varying amounts of dark-colored, caseous material. In the heart, there was a large amount of pericardial fluid (hydropericardium) consisting of approximately 2,000 ml of clear, tan-colored fluid. Impression smears from the lungs revealed numerous bacteria and frequent non-septated.
fungal hyphae. Other examined organs were within the normal limits.

**Histopathological Findings:**
In the lungs there was a severe degree of necrotizing pneumonia associated with suppurative bronchopneumonia along with frequent cavitations. The bronchi and alveoli were markedly dilated and packed with abundant purulent and mucopurulent exudates consisting of neutrophils, macrophages and cellular debris, as well as numerous bacteria colonies. Inside the pulmonary cavities, there were various degrees of lining zonal bacterial colonies embedded in the necrotic tissues, which were surrounded by numerous fungal hyphal elements. Under PAS and GMS staining, the hyphae were seen to be 30 to 40 μm in diameter, infrequently septated, thin-walled and tangled, and usually demonstrated a variable pattern of branching. Lymph nodes had severe lymphadenitis. The liver showed marked congestion, with multi-focal areas of cell swelling, in addition to slight to moderate periportal lymphocytic infiltrations. The spleen showed severe congestion, sub-capsular multi-focal hemorrhages and moderate lymphoid depletion, in addition to diffuse extramedullary hematopoiesis represented by the presence of erythroblasts and megakaryocytes. Both kidneys showed slight congestion. In the stomach, the lining mucosa of the different gastric compartments showed multi-focal hemorrhages extending to ulcers in the third stomach, with frequent bacterial colonies seen on the surface of the ulcerating mucosa as well. The small and large intestines showed chronic enteritis with moderate to severe lympho-plasmacytic infiltrations in the lamina propria.

**Immunohistochemistry:** The hyphal element in the lung lesions revealed strong and uniform positive reactions in the cell walls of the hyphae with Anti-*Rhizomucor*, but was negative for Anti-*Aspergillus* and Anti-*candida albicans*.

**Fungal cultures and identification of the fungus:** A zygomycosis, *Cunninghamamella bertholletiae*, was identified by morphological characteristics (Kwon-Chung and Bennett 1992) and finally confirmed by molecular techniques using the internal transcribed spacer (ITS) region of the ribosomal DNA (Kano et al. 2011). Comparative sequence analyses of the clinical isolate with the ITS region in GenBank showed that its queried sequence was 100% identical to *C. bertholletiae* (GenBank accession number FJ345351).

**Microbiological analysis and identification of the isolated bacteria:** Bacteriology testing for aerobic and anaerobic microorganisms yielded *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Pseudomonas oryzihabitans* in the lungs.

**Diagnosis:** A case of pulmonary zygomycosis with *Cunninghamamella bertholletiae* in a killer whale (*Orcinus orca*)

**Discussion:** Fungi may be primary or secondary pathogens that emerge during any chronic infectious process in cetaceans. Based on histopathology findings, possible causes of death include necrotizing pneumonia, pulmonary cavitations, and severe purulent bronchopneumonia with presumptive subsequent septicemia caused by opportunistic microflora. It is possible that septicemia and toxemia could cause general congestion and systemic multi-focal degenerative areas. Pulmonary cavitations and necrosis are suspected to be caused by *Cunninghamamella bertholletiae*. The present case may possibly be the first report of pulmonary mycotic infection associated with order Mucorales, *Cunninghamamella bertholletiae* sp. combined with severe suppurative bronchopneumonia which appeared to have been caused by *Proteus mirabilis*, *Pseudomonas (P) aeruginosa* and *P. oryzihabitans*.

The most outstanding pathological findings involving *C. bertholletiae* have been described as angioinvasion accompanying hemorrhages and necrosis. The primary site of zygomycoses infection usually involves the subcutaneous tissues and skeletal musculature; however, the
lungs, nasal sinus and alimentary tract are obvious entry portals for the fungus. Gastric ulcerations may have facilitated the invasion of the fungus as reported in a harbor porpoise.

Mycoses in cetaceans may be indicative of immunosuppression. Stressful conditions, including a large number of stones, ulcerations of the third stomach and transportation might be putative factors that facilitate mycosis. In the present case, there was a striking abnormality in the stomachs, in the form of a large amount of stones being present, especially in the first and second gastric compartments. A heavy load of stones could be a source of continuous mechanical injuries, physical pressure, hemorrhages, gastritis, and gastric ulcerations, as well as circulatory disturbances.

References:
Case 4
Mycobacterium Tuberculosis in a Captive Asian Elephant (*Elephas maximus*)

Singh Dinesh 1, Kamal Gaire 2, Jeevan Thapa 3

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**Introduction:** Tuberculosis (TB) is an ancient disease of animals and humans. TB has been reported in captive elephants worldwide. *Mycobacterium tuberculosis* (*M. tb*) is the predominant disease-causing agent in elephants. Clinical signs, characteristic of TB were described in Asian elephants by Ayurvedic physicians over 2000 years ago. Both African and Asian elephants are susceptible to TB although the disease appears to be more common in Asian elephants. Because *M. tb* is primarily a human pathogen, exposure to infected humans is the most likely source of infection for elephants and other humans. We examined a case of *M. tb* that occurred in a captive Asian elephant and diagnosed by serology, PCR and culture, and tried to describe the gross and histopathological characteristic of lesion.

**Clinical History:** A 45 year-old female elephant from Chitwan national wildlife park in Nepal was suffering from continuous weight loss, weakness and ventral edema died suddenly and submitted for necropsy. Animal showed positive for tuberculosis by serological test (ElephantTB STAT-PAK® Assay).

**Gross Findings:** Extensive caseo-calcareous and cavitating lesions were seen throughout the lungs associated with pulmonary abscesses.

**CASE RESULT:**

**Histopathological Findings:** Histopathological examination typically showed well demarcated epithelioid granulomas in the pulmonary lesions. The central caseous necrotic area was surrounded by inflammatory cells consisting predominantly of lymphocytes and epitheloid cells. Acid fast staining detected acid fast bacilli in central areas of caseation in the lungs.

**Diagnosis:** Mycobacteriosis (Tuberculosis)
Molecular Diagnostics: The lung sample was cultured at National Anti Tuberculosis Association center (NATA) which showed positive for mycobacteria but was unable to identify species. The real time PCR test was done on culture sample at Center for Molecular Dynamics Nepal (CMDN) which showed M tuberculosis.

Discussion:
Elephants are most likely to contract tuberculosis subsequent to contact with infected humans. Naturally-occurring tuberculosis has not been reported in free-living Asian elephants. Signs attributed to tuberculosis in elephants are mostly non-specific, e.g. inappetance, weight loss, reluctance to do strenuous work and occasionally, subcutaneous ventral edema which are seen in this case. More typically, premonitory ante-mortem signs are absent. The major pathological changes in elephants with M. tuberculosis occur primarily in the lungs and thoracic lymph nodes with lesser involvement of extra-thoracic sites. In the less extensive cases, firm granulomatous nodules, sometimes with caseous foci, are noted in the bronchial lymph nodes and pulmonary tissue. Thorough post mortem examination was not done in this case which might lead to not finding lesion in other tissue except lungs. Elephants with extensive involvement of both lungs usually die with severe caseo-calcareous and cavitating lesions. These often result in large pulmonary abscesses. Characteristic histological findings include epithelioid granulomas with some giant cell formation in the initial lymph node and pulmonary lesions and extensive caseous and pyogranulomatous pneumonia in the advanced forms. In this case except giant cell similar histopathological findings were seen in lungs. Although sparsely distributed, acid-fast bacilli are more easily found in central areas of caseation in the lungs, rather than in the lymph nodes where they are typically rare. In present case also extensive acid fast bacilli were seen in lesion.

The diagnosis of TB in elephants has been problematic. Radiography is not feasible and many techniques commonly used in humans (sputum examination and culture, intradermal tuberculin testing) are unreliable in elephants. Serological tests like ElephantTB STAT-PAK® Assay and Multi-Antigen Print Immunoassay (MAPIA) opens the door to testing in range countries. The ELISA measures antibodies against specific antigens but not available commercially. A positive AFS (acid fast stain) is suggestive of TB but not definitive. Molecular technique like Nucleic Acid Amplification Techniques and Restriction Fragment Length Polymorphism(RFLP) is there but not easily available. Our wildlife resort and national parks have a public health concern since close contact between mycobacteriosis-susceptible animals and humans. Infected elephants pose the greatest risk to mahouts who live in close daily association. Therefore, more attention needs to be paid to earlier diagnosis of Tb in elephant and treatment so that risk to human and other animal can be reduced.
References:

Case 5