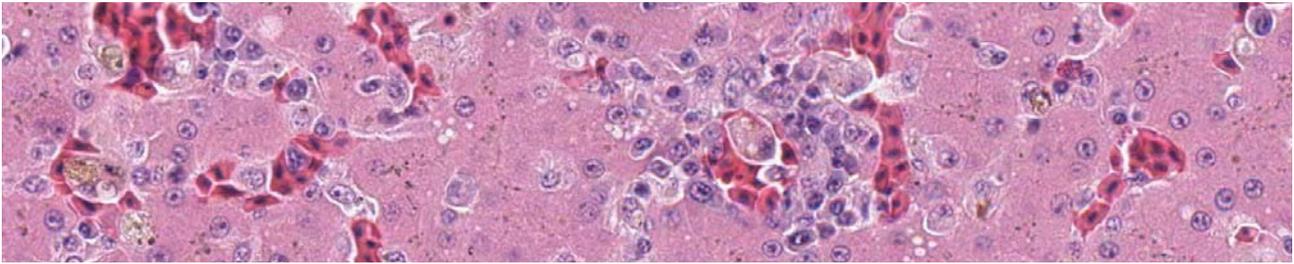


SPECIAL SESSION

Asian Zoo/ Wildlife Histopathology and Parasitology Conference, 2009



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 Seoul. 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 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 in Indonesia (2008), all conference participants were able to take a look some of cases at Web at the National Taiwan University. Thank you.

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



Case Signalment
3rd International Workshop of Asian Society of Zoo and Wildlife Medicine, 2009 Seoul
August 19, 2009

Case No.	Presenter	Institution	Slide No.	Signalment
Case 1	Dr. Chun-Ho Park	<i>Kitasato University</i>		Japanese hare (<i>Lepus brachyurus angustidens</i>)
Case 2	Dr. Theerayuth Kaewamatawong	<i>Chulalongkom University</i>	7P323T/3	Malayan tapir (<i>Tapirus indicus</i>)
Case 3	Dr. Yu-Xing Ding	<i>National Taiwan University</i>	NTU08-527E	Fat-tailed Dwarf Lemur (<i>Cheirogaleus medius</i>)
Case 4	Dr. Mami Murakami	<i>Gifu University</i>		Gentoo penguin (<i>Pygoscelis papua</i>)
Case 5	Dr. Yoon-Seok Roh	<i>Chonbuk National University</i>		Crocodile (<i>Crocodylus porosus</i>)
Case 6	Dr. Dae-Yong Kim	Seoul National University		Tiger (<i>Panthera tigris tigris</i>)

Chairpersons: Achariya Sailasuta (Chulalongkorn University) and Dae-Yong Kim (Seoul National University)

Case Diagnosis
3rd International Workshop of Asian Society of Zoo and Wildlife Medicine, 2009 Seoul
August 19, 2009

Case No.	Presenter	Institution	Slide No.	Diagnosis
Case 1	Dr. Chun-Ho Park	<i>Kitasato University</i>		Tularemia
Case 2	Dr. Theerayuth Kaewamatawong	<i>Chulalongkom University</i>	7P323T/3	
Case 3	Dr. Yu-Xing Ding	<i>National Taiwan University</i>	NTU08-527E	Acute myelomonocytic leukemia
Case 4	Dr. Mami Murakami	<i>Gifu University</i>		Avian malaria
Case 5	Dr. Yoon-Seok Roh	<i>Chonbuk National University</i>		Mycobacteriosis
Case 6	Dr. Dae-Yong Kim	Seoul National University		Amyloid producing odontogenic tumor

Chairpersons: Achariya Sailasuta (Chulalongkorn University) and Dae-Yong Kim (Seoul National University)

Chun-Ho Park, DVM, PhD.

Department of Veterinary Pathology, School of Veterinary Medicine, Kitasato University, Towada, Japan

CASE HISTORY:

Signalment:

An adult male Japanese hare (*Lepus brachyurus angustidens*)

Clinical History:

An adult male hare (*L. b. angustidens*), weighing 2.6 kg, was discovered in a moribund condition in the bush in the mountains of Aomori prefecture in Japan on May 24, 2008. It did not run away when approached. Upon manipulation, only slight falling off was observed. Shortly thereafter, the hare ran into the woods. When the observer returned to the same site, the recumbent hare was found. Although it was breathing and had a weak pulse, it soon stopped breathing and died. Upon gross inspection, many ticks were found on the neck and the external ear regions, and more than half the ticks contained ingested blood. A V-like laceration was observed on the left external ear. The skin around the tick bite wounds was alopecic and mildly thickened.

Gross Finding:

At necropsy, marked enlargement of the spleen (10 x 2 x 1 cm), enlarged cervical lymph nodes (1.5 x 1 x 0.5 cm), and many white spots on the liver, spleen, lymph nodes, and bone marrow were observed. The borders between the cortex and medulla of the spleen and the lymph nodes were not clear. The lungs were edematous and a foam-like secretion was retained in the bronchi, and one well-demarcated nodular lesion (0.7 x 0.7 x 0.5 cm) was present in the right anterior lobe. The pulmonary lymph nodes were mildly swollen.

CASE RESULT:

Histopathological Findings:

Histologically, there were tick bite wounds in the primary lesion, accompanied by chronic necrotizing granuloma with bacterial infection. In the skin of the cervical and external ear, heterophils, lymphocytes, plasma cells, and multinucleated giant cells had infiltrated the dermis to the subcutis, and had sometimes formed cystic lesions surrounded by connective tissue. The centers of the cysts were filled with red blood cells, plasma material, and cell debris. Bacterial colonies were occasionally observed within the keratin layer of the skin. In the stroma, collagenolysis, edema, and hemorrhage were observed. Multiple bacterial colonies were found within and outside the small vessels. There were no histological changes in the ticks, but bacterial colonies were observed not only in the ingested blood but also in the cavity of the intestine without blood. The blood was hemolytic. In contrast to the skin, the lesions on the visceral organs (liver, spleen, lymph nodes, lungs, and adrenal glands), brain, and bone marrow showed acute necrosis but mild or absent inflammation. Liver changes presented as multifocal acute necrosis with an irregular outline, especially near the portal vein. The lesions contained amorphous cell debris, necrotic hepatocytes, and mild infiltrations of lymphocytes and heterophils. Multiple bacterial colonies were observed in the hepatoid sinus, necrotic foci, and the cytoplasm of hepatocytes and Kupffer cells. Hepatocytes that contained bacteria were swollen to 2-3 times the size of uninfected hepatocytes. The spleen and cervical and pulmonary lymph nodes showed massive necrosis of both the white and the red pulp. Many bacteria similar to those in the liver were observed as free cells or colonies in the necrotic foci and in the cytoplasm of heterophils and macrophages. Bacterial thrombi were occasionally

observed in the lymph nodes. Diffuse pulmonary edema and localized necrotizing lesions were seen in the lungs. Multifocal necrosis with bacteria were found in the cortex of the adrenal glands, but no inflammatory reaction was observed. In the brain, multifocal necrosis, with hemorrhage and bacterial colonies, was observed in the cerebral cortex and midbrain. Multifocal necrosis with bacterial colonies was observed in the bone marrow. There were no histopathological changes in the other organs, including the spinal cord.

The bacteria were clearly stained by reticulin silver impregnation stain and Giemsa stain, but were negative for Gram stain.

Immunohistochemistry:

Most of the lesions in the skin, liver, spleen, lymph nodes, lungs, adrenal glands, brain, and bone marrow were positive for *F. tularensis* antigen. The bacteria were seen as rods or granules in the cytoplasm of heterophils, monocytes, macrophages, and hepatocytes, and sometimes formed antigen aggregates. Antigen-positive granules also were seen in the cavities of the small vessels and in the cytoplasm of vascular endothelial cells, free or as aggregates. In the ticks, scattered and aggregated antigen-positive cells also were observed in the pool of ingested blood and in the cavity of the intestine, which did not contain blood. No immunostaining was seen in the cytoplasm of the intestinal epithelial cells, salivary glands, or genital organs of the ticks.

Electron Microscopic Findings:

By electron microscopy, bacteria were found in the cytoplasm of monocytes, macrophages, heterophils, and hepatocytes. They were round to rod or almond-like in shape, and measured 200-700 nm in length. The bacteria had well-defined borders along the center and their margins. Most bacteria were enclosed by a phagosomal membrane and the others were located in the cytoplasm without a membrane. The centers of the bacteria showed high electron density and were surrounded by electron-lucent zones.

Results of PCR:

The characteristic biological properties of the bacteria were similar to those of *Francisella tularensis* subsp. *holarctica*. The results of PCR, the organism was finally identified as *F. tularensis* subsp. *holarctica*.

Discussion:

The infection is often transmitted by arthropods, including ticks, biting flies, and possibly mosquitoes, but it can also be acquired orally, via the respiratory route, by the bites of infected vertebrates, or from direct contact with infected tissue. In the present study, the route of transmission of *F. tularensis* to the hare was not identified, but the cutaneous lesions caused by tick bites were more chronic than those in the visceral organs, and bacterial antigens were detected in both the blood-injected and the noninjected ticks. The cervical lymph nodes were markedly more swollen than the other lymph nodes at necropsy. It is common that the lymph nodes draining the infection site become swollen. Therefore, we assumed that the primary lesions were formed on the skin by tick bites, and that the bacteria in the intestines of the ticks were transmitted to the skin of the hare, and then rapidly spread, either hematogenously or lymphogenously, to the cervical lymph nodes and multiple organs, and infected hare died by acute septicemia.

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Case Number: 2

3rd International Workshop of ASZWM, 2009 Seoul

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CASE HISTORY:

Slide Number: 7P323T/3

Signalment:

A 17-year-old, 320 kg, female captive Malayan tapir (*Tapirus indicus*)

Clinical History:

A 17-year-old, 320 kg, female captive Malayan tapir from a zoo in Bangkok, Thailand was referred for treatment of dental tartar and inflammation. She had inappetite, weight loss and hypersalivation. After anesthesia for the treatment, she died with sign of panting.

Gross Finding:

Necropsy showed marked enlargement of mediastinal lymph nodes, diffuse military firm to hard white nodules were found in the lung, diaphragmatic muscle, liver, spleen, kidney and intestine. Sectioned surface of these tubercles revealed white to yellowish, solid and dry necrotic center.

Yu-Xing Ding, DVM, MS; Victor Fei Pang, DVM, PhD; S.H. Vincent Hsiao, DVM, PhD;
Chian-Ren Jeng, DVM, PhD.

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CASE HISTORY:

Slide Number: NTU08-527E

Signalment:

A 13-year-old, female, Fat-tailed Dwarf Lemur (*Cheirogaleus medius*).

Clinical History:

The animal was found dead by the keeper during daily visit without any previous clinical signs.

Gross Finding:

There was bloodstain at right cheek and ear but without the sign of trauma. The spleen was enlarged with rounded, uneven edges, and firm texture. There was also a nodular projection occupying approximately one quarter of spleen. On the cut surface of spleen, a peripheral, dome-shaped region with white discoloration accounted approximately 15-20% of spleen was enclosed by a band of dark red area. In cerebrum, several small and randomly-distributed foci of hemorrhage were present in both gray matter and white matter. The lungs had diffusely reddish to shining appearance, elastic to slightly firm and also wet texture with rounded edges. The liver was enlarged with blotchy yellowish discoloration. The adrenal glands were diffusely reddish on the cut surface.

CASE RESULT:

Microscopic Findings:

Accumulations of atypical cells are present in bone marrow and in vascular structures throughout the spleen, liver, cerebrum, cerebellum, heart, lungs, pancreas, intestines, kidney and adrenal glands. In the disorganized bone marrow, there is marked hypercellularity with only 1-3 megakaryocytes. In 20 random fields, the M:E ratio is approximately 4:1. Granulocytic component including predominately myelocytes, metamyelocytes, eosinophils myelocytes, and some mature eosinophils accounts about 63 % of non-erythroid cells. The ratio of mature neutrophils to non-erythroid cells is around 6%. Monocytic component containing mainly monoblasts and some mature monocytes accounts approximately 26% of non-erythroid cells. The proportion of macrophages which sometimes engulf erythrocytes to non-erythroid cells is about 4%. Prominent erythroblastic islands and erythroid precursors are relatively inconspicuous.

In periphery blood, peribronchial arterioles and alveolar capillaries of the lungs are engorged with monotonous atypical cells. These cells are round to oval with scant to moderate amount of eosinophilic and homogeneous cytoplasm and round, oval or reniform, hyperchromatic, central to eccentric nuclei. The nuclei contain vesicular chromatin with inconspicuous nucleoli. There are mild anisocytosis, and moderate anisokaryosis and a low mitotic rate, ranging from 0-2 per 400× field.

In cerebrum, variable numbers of the atypical cells are present in the vasculature with multifocal foci and exterior garland band of hemorrhage surrounding damaged blood vessels. In spleen, white pulp follicles are displaced and effaced, and areas of red pulp are occupied by a large numbers of monotonous atypical cells accompanied with locally-extensive necrosis and hemorrhage. In liver,

crowded accumulations of monotonous atypical cells are noted within portal triads, central veins and sinusoids.

Immunochemical Staining:

The atypical cells are partially immunopositive to both CD3 and lysozyme, while immunonegative to CD79a.

Berlin Blue Staining:

Positive reactions, characterized by accumulations of intracellular (cytoplasmic) or extracellular, dark blue to purple granules, were detected multifocally in astrocytes and oligodendrocytes of the cerebral gray matter, in portal triads, hepatocytes, and sinusoids of the liver.

Morphological Diagnosis:

1. Acute myelomonocytic leukemia, involves spleen, liver, cerebrum, cerebellum, heart, lungs, pancreas, intestines, kidney and adrenal glands, Fat-tailed Dwarf Lemur
2. Hemosiderosis, mild to moderate, cerebrum and liver, Fat-tailed Dwarf Lemur

Comments:

Fat-tailed Dwarf Lemurs (*Cheirogaleus medius*) are endemic animals in Madagascar. They are usually omnivores, nocturnal and female-dominant animals. The storage of fat in their tails provides the source of energy during the hibernation.

In past years, the iron storage disease of captive lemur was extensively studied while the incidence of neoplasia was rare reported. Few papers of tumors have been documented in lemurs, including a mixed epithelial and stromal tumor of the kidney, interstitial cell tumor, spontaneous cholangiocarcinoma and T-cell-rich B-cell lymphoma. However, there is only one case regarding lymphoproliferative disorder in lemur so far.

In the previous study, the diagnosis of tumor origin could still be achieved by the aid of immunohistochemical staining, such as the use of CD3, CD79a, lysozyme, cytokeratin, vimentin, and BLA36. The underlying causes of lymphoma/leukemia in lemurs are still unknown. However, the infection of simian T-lymphotropic virus (simian T-cell leukemia virus), gibbon ape leukemia virus, oncogenic herpesviruses, and type C, D, and E retroviruses are associated with leukemia and lymphomas in captive non-human primates. In addition, the incidence of T-cell related lymphoproliferative disorder seems to be higher than B-cell or myeloid originated disorders in non-human primates.

The infection of herpesvirus simplex (*Herpesvirus hominis*) has been reported in captive lemur in 1992. The serology study of simian immunodeficiency virus (SIV) and herpes simplex virus type 1 (HSV-1) was carried out in free-ranging ring-tailed lemurs in Madagascar in 2007. The results showed none of studied lemurs have antibodies against HSV-1 and only five had antibodies reacted with SIV. This revealed the viral infection may not act as an important influence on lemurs.

The iron storage disease in monkeys has been firstly reported since 1960s and consistently found in captive lemurs. Lemurs kept in captivity have been reported to be highly prone to accumulate excessive amounts of iron in tissues, including cerebrum, duodenum, liver, kidney, and spleen. *Cheirogaleus medius* was regarded as one of lemur species which was prone to the iron deposition in a retrospective study in 2006. Iron plays an essential role in all living animals but also in stimulating tumor cell proliferation and grow. The tumor cells obtain more iron by upregulating transferrin receptor 1 (TfR1) and increasing rate of iron uptake from transferrin, such as most clonogenic leukemia cells and proliferating malignant B lymphocytes expressing increasing transferrin receptors. Therefore, animals receiving excessive dietary iron are associated with

enhanced tumor cell growth.

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CASE HISTORY:

Slide Number:

Signalment:

An 11-year-old male Gentoo penguin (*Pygoscelis papua*)

Clinical history

An 11-year-old male Gentoo penguin (*Pygoscelis papua*) refused food for 3 days, and then showed severe depression with complete loss of activity, lying face down throughout the day. While blood was being drawn for testing the animal collapsed, and died shortly thereafter. The penguin was part of a flock in a zoo.

Gross findings

Grossly, the body was normal in size, with a moderate amount of subcutaneous fat. The liver was slightly enlarged and congested, with multi-focal white spotty foci on the surface. The spleen was also slightly enlarged and congested. There were frequent pinpoint hemorrhages in the mucosa in the small and large intestine.

CASE RESULT:

Histopathological Findings:

There were *Plasmodium*-like protozoa organisms in the liver, spleen, lungs and heart. In the liver, there were moderate to severe degrees of inflammatory reactions consisting of lymphocytes, plasma cells, heterophils and macrophages. The macrophages frequently contained a larger amount of brown pigment and had infiltrated around the Grisson's sheath and central veins. In the sinusoidal capillaries, frequent macrophages and Kupffer cells contained schizonts. Each of the schizonts contained numerous merozoites. Parasitic organisms were also observed in the hepatic cells. In the lung, vacuolated macrophages containing the schizonts were frequently observed in the parabronchi or in the capillaries. These protozoan organisms stained bluish in Gimsa stain. The spleen had a slight degree of extramedullary hematopoiesis and occasional schizonts in the macrophages. The heart had pericardial hemorrhages and infiltration of macrophages with schizonts.

In the intestine, there was chronic enteritis with prominent lympho-plasmacytic infiltrations as well as frequent focal hemorrhages in the lamina propria.

Diagnosis: Avian malaria suspected

Discussion:

Based on the frequent presence of *Plasmodium*-like protozoan organisms in various organs, including the liver and lungs, the present case was diagnosed as avian malaria. The gross findings revealed no characteristic change to be identified, as in previously reported cases involving penguin. Clinical features, including sudden anorexia and decreased activity may be common clinical

findings in penguin malaria, although these are non-specific signs. *Plasmodium sp.* protozoan organisms frequently contained many merozoites, mainly in the macrophages found in the liver, spleen, and lungs. In the liver, we found pigmentation as well as infiltrations of inflammatory cells, which were similar to those reported in cases of avian malaria involving penguins.

This disease is highly infectious among birds, especially penguins, and in some cases an acute clinical course with a high mortality rate has been observed. The present penguin demonstrated symptoms for only three days before collapsing and dying. Malaria infection should be considered if the penguin dies suddenly following a rather short clinical course, and hepato-splenomegaly is observed at necropsy.

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CASE HISTORY:

Slide Number:

Signalment: Crocodile (*Crocodylus porosus*)

Clinical History:

During January 2008, five crocodiles died suddenly with no previous clinical symptoms in different dates and submitted for necropsy.

Gross Findings:

Fluid accumulation in the right pleural cavity and white miliary nodules were observed in the right lobe of lung parenchyma.

CASE RESULT:

Histopathological Findings:

Histopathological examination typically showed well demarcated granulomas. The central necrotic area was surrounded by inflammatory cells consisting predominantly of lymphocytes and multinucleated giant cells. Lymphocyte infiltration was also found in the liver, stomach, and small intestine. Fite's acid fast staining detected unbranching bacilli in the central area of multinucleated giant cell.

Diagnosis: Mycobacteriosis (Tuberculosis)

Molecular Diagnostics:

The diagnosis of mycobacteriosis was confirmed by nested PCR using *mycobacterium* genus specific primers with formalin-fixed, paraffin-embedded lung tissue. Sequence analysis of the amplicon DNA showed that the species of *mycobacterium* shared a 98% homology with the gene encoding the hsp65 gene of *Mycobacterium szulgai*.

Discussion:

Mycobacterium szulgai is an uncommon mycobacterium species and elicits granulomatous inflammation in any organ including lungs. Its pathogenicity in immunodeficient human patients has been well documented, but reports of *Mycobacterium szulgai* infection associated disease in animals are rare. Mycobacteria usually reside in soil and aquatic habitats, and most of them are saprophytes. Captive reptiles are frequently mismanaged and exposed to circumstances that may repress the immune system. Hence, immunosuppressed reptiles are vulnerable to mycobacterium infection and infected reptiles themselves could represent an additional reservoir for humans. Zoos have a public health concern since close contact between mycobacteriosis-susceptibel animals and humans. Therefore, more attention needs to be paid to this important zoonotic disease.

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CASE HISTORY:

Slide Number:

Signalment: A 13- year-old male tiger (*Panthera tigris tigris*)

Clinical History:

A 13- year-old male tiger (*Panthera tigris tigris*) that has been kept in the Everland Zoological Garden, Korea. The tiger was euthanized due to poor prognosis after suffering from extensively growing mass at the gingiva.

Gross Findings:



CASE RESULT:

Histopathological Findings:

Histologically, the neoplastic mass is consisted of odontogenic epithelial cells with multiple nodular amyloid deposits as stained by Congo Red. In the area of cyst formation, lining epithelium showed squamous metaplasia with keratin production. Dystrophic calcification, smaller foci of irregular basophilic material with spheroidal and laminated structure, so called Liesegang rings were also noted.

Immunocytochemical Results:

Immunohistochemically, all the neoplastic epithelial cells were strongly positive for cytokeratin and negative for vimentin.

Diagnosis: Amyloid producing odontogenic tumor

Discussion:

Epithelial odontogenic tumors are uncommon in domestic animals and most of the reported cases being dogs. One subtype, so called as amyloid producing odontogenic tumor (APOT, previously

called calcifying epithelial odontogenic tumor) is generally located in the mandible with tendency to be slow growing but occasionally causes bone destruction and displacement of teeth. Histologically, the neoplasm is characterized by epithelial proliferation, mineralization in the epithelium and stroma, and deposition of amyloid.

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