

Comparative Pathology Case 8

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Clinical History: A 5-year-old female white pigeon was presented to The Animal Medical Center because of blood on the feathers of the left wing. Physical examination revealed a 1 cm raised dark fluctuant mass on the base of the left wing. Mesenchymal neoplasia was diagnosed by fine needle aspiration due to the presence of pleiomorphic spindle cells with a high nucleus-to-cytoplasm ratio and prominent nucleoli.

Radiographs of the bird demonstrated extensive destruction and a widened bone contour of the proximal humerus and an irregular soft tissue swelling with cloud-like density pattern and undermineralization of the midshaft and distal humerus (Figure 1) . All long bones revealed an ivory density pattern with irregular pneumonized spaces (air sacs) in the right humerus and scapulae.

Diagnosis: Synovial sarcoma in the left scapulohumeral and coracoid joints of a 5-year-old white pigeon.

Gross Findings: The left wing and scapula were amputated and submitted for pathologic examination. Gross examination revealed invasion and replacement of the proximal humerus by a pinkish-gray cystic mass. The lesion extended into the adjacent soft tissue.

Histopathological Findings: Histologic examination of the scapulohumeral joint revealed a poorly delineated infiltrating mass that formed villous and papillary projections and interlacing bundles punctuated by cleftlike spaces that invaded adjacent soft tissue, muscle bundles and bone. Cells were polygonal-to-spindle with indistinct cell borders and scant pale basophilic cytoplasm. Neoplastic synovial cells were admixed with hyperplastic air sac epithelial cells, including a few columnar ciliated cells(Figure 2). There were marked multifocal necrosis and hemorrhage in and around the neoplastic mass.

Immunohistochemical Results: Neoplastic cells were decorated by antibodies to vimentin, S-100, and keratin using the ABC method of immunochemical staining.

Electron Microscopic Findings: The neoplastic tissue consisted of the fusiform and spindle cells in the sarcomatous region, and the epithelial cells ultrastructurally resembled undifferentiated mesenchymal cells in the other regions.

Discussion: The clinical findings of swelling, pain, and limited motion of the scapulohumeral and coracoid joints in this pigeon are similar to those in the affected joints of dogs with synovial sarcoma. Radiological findings and gross features of this avian synovial sarcoma are similar to those found in humans and dogs with synovial sarcoma.

The histologic features and immunohistochemical reactions of this avian synovial cell sarcoma are identical to those described for synovial cell sarcoma in canine and human patients. The spindle cell component was reactive to antibodies to vimentin and the epithelioid cells were reactive to antibodies to keratin. (Figure 3) S-100 staining of the spindle cell component has been seen in some cases of synovial sarcoma in humans and dogs.

The air sac represents an extension of the pulmonary alveoli. These alveoli are composed of a thin membranous bag extending between the viscera, the muscles, into the bone, and under the skin, acting to significantly diminish the specific gravity.

Diagnostic Criteria:

- 1.Fusiform, spindle cells, and or epithelial cells formed villous and papillary projections.
- 2.Immunohistochemical reaction.
- 3.Ultrastructural findings.

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Comparative Pathology Case 9

Contributor: F I Wang, DVM, PhD

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Clinical History: Progressive distension of the abdomen has been noticed on this 7-month-old female domestic cat (Figure 1 & 2) since she was adopted by the owner 3 months ago.

Diagnosis:

1. Perinephric pseudocyst, unilateral, left kidney.
2. Pyelocalyceal diverticula, polar with extension to mid zone, unilateral, left kidney.

Gross Findings: A round white cyst, around 8 cm in diameter, with a 5-cm wide rupture, completely invested the left kidney (Figure 3) . Outside the renal parenchyma, there existed a large cavity encircled the kidney along its craniomedio-caudal border. The cyst wall was leathery, thick, composed of yellow outer and inner layers with a white middle layer. The cavity surface had several variably size reddened areas while the rest of it was yellowish and roughened. The cavity contained within it fluid, fat and blood vessels. During the surgical procedure, 230 ml of clear, slightly reddish, odorless fluid was recovered from the abdominal cavity of this 3.2 kg kitten.

Reticulated white spaces were noticed on both poles of the kidney. Most of these spaces appeared to communicate with major calices and the pelvis. These spaces also extended outward compressing the parenchyma against the thickened renal capsule (or cyst wall).

Histopathological Finding: A cavity was present within the renal fascia and outside the renal capsule. The cyst wall was hypertrophied, composed of densely fibrosed outer (renal fascia) and inner layers with a loosely fibrosed middle layer (capsular adipose) which contained focal collection of lymphocytes (Figure 4) . The identity of the inner layer was uncertain. Vessels on the cyst wall, particularly those on the inner border of capsular adipose, were severely congested, sometimes thrombosed, with local extensive areas of hemorrhages into the rupture site. The

cavity was not lined by epithelium and it contained adipose tissue, dilated and congested vessels, dilated lymphatics, arteries with surrounding thick and dense fibrous tissue, extravasated RBCs, nerves, etc.

Most of the renal parenchyma were replaced by folded spaces (diverticula) lined by transitional cell epithelium. Most diverticula were continuous with major calices and the pelvis. Along the border of diverticula, a few surviving mature renal corpuscles and tubules were present together with fibrosis and diffuse interstitial mononuclear cell infiltration. Outside the pelvis and in the hilus area, hemorrhages were also noticed (not in the submitted section).

Discussion: Pseudocysts may be classified on the basis of their fluid content: (1) Perinephric extravasation of urine, (2) Perinephric hematoma, (3) Perinephric lymphocele, and (4) Perinephric pseudocyst of unknown origin. The hemorrhages were fresh and there was no endothelial lining. Due to its close proximity to the medial border, this pseudocyst was likely to be in category (1), although the variety of contents within the cavity seemed to suggest a more complex origin.

Pyelocalyceal diverticula may be classified into Type I involving minor calyx or infundibulum with a polar location in the kidney rarely causing clinical symptoms; Type II involves renal pelvis or adjacent major calyx with a mid zonal location, commonly causing clinical symptoms. Due to their extensive involvement, these diverticula are probably a mixture of both types.

Unilateral concurrence of pyelocalyceal diverticula with perinephric pseudocyst has not been reported in cat. Progressive abdominal distension seen clinically seems to suggest a communication between the diverticula and the pseudocyst. The potential causes for pseudocysts include trauma, surgery, neoplasia, venous congestion, and hypertension. Affected cats are usually old and have concomitant chronic renal disease. Interestingly, this kitten had a rather early onset at the age of 4 months. A congenital cause or trauma is suspected, but cannot be confirmed.

Several attendees have mentioned the possibility of this pseudocyst being part of a hamartoma. Their suggestion is certainly highly appreciated, although we feel that the majority of the perinephric space is occupied by contained fluid which we believe is beyond that could be generated by circulatory disturbances subsequent to hamartoma. We feel that before further evidence come to light, the term "pseudocyst" still faithfully describe current studied subject.

Diagnostic Criteria:

Pseudocysts - no epithelial lining

Pyelocalyceal diverticula - transitional cell lining

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Comparative Pathology Case 10

Contributor: Y S Ho, MD, MS

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Clinical History: A 7-year-old girl suffered from intermittent RUQ pain for 2-3 years, which had aggravated in recent month. The characteristics of the pain were dull in nature, gradual onset, 5-10 minutes in duration, and several days in once. The pain was aggravated by bending the body, exercise, and blunt trauma; it was ameliorated by rest. She received abdominal ultrasonography and endoscopic retrograde cholangiopancreatography (ERCP) which showed an anomalous and common channel of the pancreatobiliary ductal system and a choledochal cyst 3.7cm in diameter (Figure 1) . The choledochal cyst was totally excised by Reux-en-Y hepatojejunostomy. The patient is well 3 years after the operation.

Diagnosis: Choledochocyst, type 1 pathologically and adult type clinically.

Gross Findings: The cyst showed fusiform dilation with opaque gray-white appearance of the walls and straw-colored in content. On sectioning, the wall was diffusely thickened 0.3 cm, about three times normal size.

Histopathological Findings: In cystic dilated regions, the was marked fibrosis and walls without lined epithelium (Figure 2) .

Histochemistry Results: Masson's trichrome stain: Numerous collagen fibrils were noted.

Electron Microscopy Findings: Ultrastructural findings of the non-dilated portions showed mild cholangitis with hypertrophy of the mucosal layers, combined with mild hypertrophy and thickening of the fibromuscular and subserosal layers. Whereas in the dilated portions a series of variable degrees of histopathological changes including mucosal sloughing (Figure 3) , splitting of intercellular junctional structures (Figure 4) , basement membrane degeneration, and rupture, submucosal vessels occlusion (Figure 5) with ischemic necrosis and fragmentation of the fibromuscular walls were seen.

Discussion: Choledochal cysts, or congenital dilations of the common bile duct, are a relatively rare abnormality first reported clinically by Douglas in 1852. O'Neill described a classification of choledochal cysts into five types, based on anatomy and cholangiographic findings. They are as follows: Type I: segmental or diffuse fusiform dilation of the extrahepatic bile duct; Type II: diverticulum of the extrahepatic bile duct; Type III: choledochoceles; Type IV: multiple cysts of the intra- or extrahepatic duct or both; and Type V: single or multiple intrahepatic cysts. Over 90% of choledochal cysts are of type I variety.

Clinically, choledochal cysts are generally classified as infant or adult type. In the infant type, babies ranging from one to three months of age show obstructive jaundice, acholic stools, and hepatomegaly, in a clinical picture indistinguishable from that of biliary atresia. In the so-called adult type of choledochal cyst, clinical manifestations generally do not become evident until after 2 years of age. It is in this group of patients that the classic triad of pain, mass, and jaundice may be present.

A number of theories have been proposed to explain the occurrence of choledochal cysts, as follows: (1) obstructive theory, (2) theory of primary weakness or abnormality of the bile duct itself, (3) theory of combination of both obstruction and intrinsic bile duct defects, and (4) common channel theory of reflux of pancreatic enzymes and dissolution of the ductal system due to an anomalous arrangement of the pancreatobiliary ductal system. On the basis of the findings of the ultrastructural alterations and ERCP features of the common bile ducts of this case; and suggest that anomalous fusion of the pancreatic duct and common bile duct, so called common channel theory; which allows the pancreatic juice to dissolve of the ductal walls, are responsible.

As the abundant Japanese literature reports, the disease is much more common in the Orient than in the West. The female-to-male ratio is 3 to 4:1. Ultrasonography is probably the best screening study, followed by ⁹⁹Tc DISIDA scintigraphy if a choledochal cyst is suspected. Roux-en-Y hepaticojejunostomy is popularly used in treatment of Type I choledochal cyst. The remaining types of choledochal cysts must be treated as indicated by their anatomy. The common complications of choledochal cyst are cholangitis with sepsis, hepatic failure, stone formation, GI tract bleeding, pancreatitis, or carcinoma.

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Comparative Pathology Case 11

Contributor: S D Chen, DVM, PhD

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Clinical History: Adult rat, Sprague-Dawley, the common bile duct had been ligated for 84 days.

Diagnosis: The common bile duct severely dilated, lining epithelial cells proliferated and invaginated into submucosa to form some glandular structures. The duct wall severely thickened. The bile ducts in liver portal triad also prominently proliferated, even extended into the liver parenchyma. Focal necrosis was found and the necrotic area was filled with erythrocytes. Both lesions in liver and common bile duct indicated that the common bile duct had been ligated for a long period.

Gross Findings: After 84 days, ligation, before the ligated site, the common bile duct dilated tremendously, filled with greenish bile, and adhered with surrounding tissues (Figure 1). The diameter of duct lumen increased and reached about 1 cm. On the other hand, the liver showed no obvious lesions.

Histopathological Findings: Bile duct proliferation is the most prominent lesion in liver. Some of them extended into parenchyma from portal triad in which edema fluid and inflammatory cell infiltration were found (Figure 2). Focal necrosis, typical coagulative type, scattered in liver, some of them full of erythrocytes. In common bile duct, the lumen was filled with yellowish bile, and the epithelium proliferated and invaginated into submucosa to form many small glandular structures (Figure 3). The duct wall was thickened by a lot of fibrous connective tissue. Besides, the pancreas was also involved, with focal necrosis and fibrosis also found in some areas. On the other hand, no lesion could be found in the lymph nodes beside the common bile duct.

Histochemical Results: Alcian blue stain was used to prove the existence of Goblet cells in common bile duct, and a positive result was obtained in the target cells.

Electron microscopic Findings: In the liver, many pathological changes were found in the necrotic area, including: nucleolar margination, pyknosis, dilated endoplasmic reticulum, degranulation of ribosomes, swelling of mitochondria, whorling of mitochondria cristae, accumulation of lipid droplets in cytoplasm. The bile canaliculi was distorted, dilated, and without microvilli (Figure 4) .

Discussion: Bile duct ligation is a very useful experimental model to study extra-hepatic jaundice; it had been used extensively in morphological and biochemical studies. Although the bile duct ligated rats showed many clinical signs and hepatic lesions, most of them could survive and recover to normalcy. Pathologic changes after bile duct ligation have been described in detail in many papers, but the mechanism of survival has not been explained yet. Therefore, I researched the ligation of the common bile duct by sacrificing the rats from 1 day to 105 days at 7 day intervals, observing the pathologic changes, and examining alanine transferase (ALT), aspartate transferase (AST), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT), choline esterase (CHOL), and total bilirubin (TBIL) with a Serum Automatic Analyzer after taking 1 ml blood from the posterior vena cava just before the rat was sacrificed. In addition, in order to confirm that the ligation had been completed, the common bile duct was injected with 1% Toluidine blue into the dilated portion until the dye appeared in the intestine, but did not pass through the posterior portion behind the ligated site.

The results of blood examination showed that, after bile duct ligation, the ALT, AST, ALP, GGT, CHOL, and TBIL promptly increased on the first day, but declined gradually to normal after day 7, persisting until day 105.

According to the data above, it could be suggested that the survival mechanism of bile duct ligated rats was the dilatation, regeneration, and recanalization of the extrahepatic bile duct, in which the bile was re-discharged into the intestine via the new tract.

Diagnostic Criteria:

- Bile duct proliferation and focal necrosis in liver
- Common bile duct dilatation and epithelial cell proliferation and invagination into duct wall
- The wall of common bile duct thickened
- Concentrated bile in common bile duct

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Comparative Pathology Case 12

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Helicobacter Gastritis: Helicobacter pylori is the causative agent of common stomach disease in human, the only known infective agent of the stomach. It is also strongly implicated in the etiology of gastric and duodenal ulcers.

Pathology of Helicobacter Infection: Helicobacter pylori causes acute gastritis, which is characterized by infiltration of neutrophils in the superficial (peri-foveolar) gastric mucosa associated with injury to the foveolar lining cells (Figure 1). The organisms colonize the surface of foveolar cells (Figure 2) and in the intercellular space (Figure 3). It is not invasive and do not invade into the lamina propria, except in the rare circumstances of immune-compromised hosts, such as AIDS patients, Chronic infection leads to chronic superficial gastritis and chronic atrophic gastritis, in which the lymphocytes and plasma cells predominate. In acute flare up of the infection, neutrophils make their appearance and the conditions are referred to as chronic "active" superficially gastritis and chronic "active" atrophic gastritis.

Helicobacter organisms, by virtue of causing chronic atrophic gastritis, are also strongly implicated in the pathogenesis of gastric carcinoma and gastric lymphoma.

Clinical Manifestations:

1. Epigastralgia.
2. Fullness of stomach, indigestion.
3. Acid regurgitation and belching of gas.

In Taiwan, Helicobacter organisms can be identified in more than 98% of gastric biopsies in the patients presented with the above symptoms.

History of The Discovery of Helicobacter pylori:

- 1920 Luck: urease in the gastric mucosa.
- 1938 Doenges: Spirochaetes in gastric glands of human.
- 1940 Freeberg and Barron: Spirochaetes in 37% of resected human stomachs.

1981 Marshall: Investigation of relation between Spirochaetes and gastritis
and successful culture of Helicobacter (Campylobacter) pylori in 1982.

Morphology of Helicobacter pylori:

1. Electron microscopy: 0.5-1.0 x 4.0 um spiral organisms with bluntly rounded end and unipolar multiflagella (4-6).
2. Light microscopy:
 - (1) Straight or slightly curved rods.
 - (2) Coccal forms under unfavorable growing conditions.
 - (3) Staining characteristics:
 - (a) Hematoxylin stain.
 - (b) Giemsa stain.
 - (c) Warthin-Starry silver stain (Figure 4) .
 - (d) Immunoperoxidase reaction with monoclonal antibodies.

Habitat of Helicobacter Pylori:

1. Highly susceptible to drying and heat.
2. Mode of transmission unclear.
3. Only colonize and infect gastric type (foveolar epithelium) mucosa, especially the antrum of the stomach. Heterotopic gastric mucosa (Meckel's diverticulum) or gastric metaplasia (duodenum) may be infected.

Treatment: Helicobacter organisms are highly amenable to Bismuth preparations, antibiotics, and sulfa drugs. Cure requires long term therapy for more than a month. Eradication of organisms is relatively difficult and recurrence is reported in 10% of cases.

Comparative Pathology Case 13

Contributor: S S Yeh, MD

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Clinical History: This patient is a 43-year-old male. He suffered from adenocarcinoma of sigmoid colon and underwent segmental resection which showed pericolonic fat invasion of the tumor with no lymph node or distant metastasis. Postoperation antibiotics treatment with Cefradine (1st generation Cephems) was initiated and lasted for 2 weeks until severe diarrhea developed. He was readmitted and frequent diarrhea (up to 20-30 times/day) persisted; meanwhile, leukocytosis (up to $68,000/\text{mm}^3$) was noted. Ascites was drained and showed yellowish turbid exudate. Vancomycin treatment (500mg, q6h, po) was started and colonoscopy was conducted with biopsy presented in this meeting. Conservative treatment was given and diarrhea subsided gradually and was cured after about one month of treatment.

Diagnosis: Colorectum---pseudomembranous colitis

Gross Findings: The mucosa showed general hyperemic change with numerous discrete small yellow-gray plaques (Figure 1). This lesion extends from rectum to almost the whole length of colon.

Histopathological Findings: The mucosal crypts were damaged and necrotic and coated with a spray of cellular debris, fibrin, mucus, and inflammatory cells forming a mushroom-like picture (Figure 2). Giemsa stain highlighted the micro-organisms which were rod-shaped and noted in the inflammatory exudate (Figure 3). Gram's stain, however, did not reveal the same micro-organism due to the small amount of specimen.

Discussion: The formation of a "pseudomembrane" on the colonic mucosa is a non-specific event which can arise in different conditions of colonic injuries such as mercury poisoning, intestinal ischemia, or in the course of severe infections. Among them, the most common and significant etiology is antibiotic treatment, especially the broad-spectrum ones. It may develop during antibiotic usage or up

to 2 weeks subsequent to the usage. The disease usually subsides some time after the offending antibiotics are withdrawn, but it is potentially lethal. Clostridium difficile is the most frequently incriminated micro-organism. This organism can exist in the colon of some healthy asymptomatic subjects, presumably in small numbers held in check by ecologic defenses. The perturbation of the local ecology by antibiotics can tip the balance toward overgrowth of Clostridium difficile which produces an array of toxins. Toxins A and B have been identified which are both cytotoxic and enterotoxic and are responsible for the histologic changes. The disease may be localized in the intercryptal surface associated with a small cluster of leukocytes, or, in the other extreme, can spread out and become confluent and cause full-length mucosal necrosis. In this case, the lesions superficially damage the crypts with inflammatory coating.

Diagnostic Criteria:

1. Suspected by plain radiographs which show an irregular mucosal outline of the colon.
2. Gross or microscopic evidence of the characteristic pseudomembranous lining of the colonic mucosa, done by sigmoidoscope and biopsy.
3. The diagnosis is confirmed by culturing clostridium difficile from the stool or toxin assay.

Comparative Pathology Case 14

Contributor: S H Lee, DVM, MS

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Clinical History: A 5-year-old male Napoleon dog with clinical signs of coughing, labored breathing on exercise, and swollen abdomen was presented at clinic. The oral mucosa was pale and slightly yellowish. Splenomegaly and hepatomegaly were detected by palpation. The dog did not improve his physical condition to a variety of treatments.

Diagnosis: Canine dirofilariasis.

Gross Findings: At necropsy, the most remarkable finding was seen in the thoracic cavity. The heart was markedly enlarged and round in shape, and contained a mass of adult heartworms tangled around the tricuspid valve and bundled up in the atrium and ventricle (Figure 1) . The pulmonary arteries were enlarged and also filled with adult heartworms (Figure 2) . The lung was edematous with marble patterns of gray and pale areas. The posterior cava was markedly dilated and fully packed with heartworms. The liver was severely congested and swollen around 3X its normal size. The spleen was small and atrophic. There were several white spots 1-3mm in diameter observed in the left kidney cortex beneath the capsule. A moderate ascites and hydrothorax was noted.

Histopathological Findings: Lung: The larger pulmonary arteries were characterized by fibrous endothelial proliferation with formation of numerous villous projections of fibrous tissue protruding into the lumen (Figure 3) . In some arteries, there were thrombi consisting of dead worm sections, and there were organized thrombi of granulation tissue adhering to the intima (Figure 4) . The smaller pulmonary arteries showed medial hyperplasia. Congestion and hemorrhage with proliferation of macrophages and infiltration of neutrophils were observed throughout the pulmonary parenchyma. A moderate number of hemosiderin-laden histiocytes were found in the alveoli. Microfilaria were seen in capillaries (Figure5) .

Heart: Focal myocardial degeneration and necrosis characterized by areas of myocytes hyalinization, coagulation, granulation, vacuolization, fragmentation, and

myocyte lysis were main lesions in the ventricular myocardium. The atrium had proliferation of fibroblast in the epicardium.

Kidney: The kidney had moderate interstitial infiltration of lymphocytes and plasma cells primarily involving the cortex. Microfilaria were observed in the capillaries.

Lymph node: The nodes were congested and there were hemosiderin depositions. Diffuse hemosiderosis was also seen in the spleen.

Liver: The portal areas had proliferation of fibrous tissue and inflammatory infiltrates, and the peripheral hepatocytes were necrotic. The central veins were thickened with proliferation of fibrous tissue. Severe sinusoid congestion was found.

Histochemistry Results: Masson trichrome stain revealed fibrous proliferation in the pulmonary arteries.

Discussion: A retrospective blood parasitic study in Taipei city area revealed that the incidence of dirofilariasis has drastically increased since 1988. Other studies indicated that canine suffering from microfilarias has increased from 5.3 to 24.2 per cent in the past ten years. There is neither sex nor age difference in dog susceptibility to dirofilariasis. However, Pit Bull Terrier may be more susceptible than other breeds (41 per cent) to the infection.

Around 70 species of mosquito have been identified as potential vectors of Dirofilariasis. The zoonotic problem of potential heartworm disease has been known for the past 30 years, and the incidence of human infection is probably greater than recognized. More investigations regarding epidemiologic heartworm infection in Taiwan are urgent.

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Comparative Pathology Case 15

Contributor: H Chiang, MD

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Clinical History: A 56-year-old housewife was admitted to the Veterans General Hospital for an asymptomatic pulmonary nodule which was discovered on a routine roentgenogram of the chest. Except for hypertension, she has no past history of illness. In the recent two years, she had twice traveling, once to the Northern America and the other to the Western Europe.

On admission, the chest roentgenogram revealed a 2.5x3 cm of "coin lesion" in the periphery of the right middle lung field (Figure 1). Bronchoscopy showed a negative finding and ultrasound-guided percutaneous aspiration of the nodule yield negative result for malignant cells or microorganisms. Under the impression of lung tumor, exploratory thoracotomy was performed. Frozen section of the lesion revealed benign. Wedge resection of the lesion was done.

Diagnosis: Pulmonary dirofilariasis

Gross Pathology: A wedge-resected piece of lung tissue containing a sharply defined grayish white mass, 2.5x2x2 cm, very close to the pleura.

Histopathology: The lesion consists of a central area of infarction surrounded by a narrow zone of granulomatous inflammation (Figure 2). A necrotic, medium-sized blood vessel was identified within the infarcted area and is filled with a recent thrombus and one cross profile of a degenerative parasite (Figure 3). The diameter of the worm measures about 250 μ m. It consists of a thick, faintly eosinophilic layered cuticle with prominent diametrically opposed internal ridges and abundant somatic muscles. The body cavity contains one digestive tract and one genital tract. The morphology is consistent with a male immature adult of dirofilaria immitis. Chronic nonspecific inflammation including moderate amount of eosinophils and focal fibrosis is present in the vicinity of the granulomatous inflammation.

Discussion: Dirofilaria immitis is a common parasite of dogs. The adult worms are coiled and formed tangled masses in the right ventricle of the definitive host.

The parasite is transmitted from animal to animal by many species of mosquitos. Man is an accidental host for D. immitis. The worms probably locate in man's right ventricle but die before reaching maturity and are thus carried into the pulmonary arteries where they impact and form the nidus of a thrombus.

Human pulmonary dirofilariasis is an important zoonosis in the United States. The disease was first reported in 1961.83 cases has been reported in the United States up to 1989. A few cases has also reported from other country such as Japan and Australia. In Taiwan this is the first and only case reported. Since the current prevalence of dirofilariasis among indigenous dogs has been reported to be 30-40%, as high as that of the United States, we have reason to speculate that this patient might get the infection at homeland. Taiwan seems to be a good environment for transmission of dirofilariasis.

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Comparative Pathology Case 16

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Clinical History: An adult female Red-Bellied Tree squirrel (Figure 1) was submitted for pathological examination by a local zoo. The animal was 1 of 6 that had been previously raised by a private owner. He died on November 2, 1992 without obvious illness.

Diagnosis: Acute fatal toxoplasmosis, Red-Bellied Tree Squirrel

Gross Findings: Many disseminated white to yellowish foci and two yellowish plaques (0.3 x 0.3, 0.5 x 0.5 cm in size) were present in the lungs (Figure 2). An increase of clear fluid was noted in both thoracic cavity and pericardial sac. The small intestine showed segmental thickness in the duodenum. In addition, the mesenteric and pulmonary lymph nodes were moderately swollen.

Histopathological Findings: Microscopically, the disseminated pulmonary lesions were characterized by multifocal necrosis and interstitial pneumonia associated with many rapidly multiplying tachyzoites of Toxoplasma gondii (Figure 3). The tachyzoites recognized in tissue sections mostly occurred in rounded and ovoid form, ranging from 2u to 4u in diameter. They were usually ingested within macrophages and free in the lesions, occasionally found in bronchiolar epithelial cells and cells lining the alveoli. Foci of necrosis involving the alveoli, bronchiolar epithelial cells, and blood vessels were scattered throughout the lesions. The alveolar septa were thickened by infiltrations of mononuclear inflammatory cells. Large numbers of macrophages filled the alveoli. Hypertrophy and hyperplasia of bronchiolar and alveolar lining epithelial cells were seen in some of the affected areas. Lesions in the liver were minute foci of coagulation necrosis and mononuclear inflammatory cells infiltrating the portal triads and around the central veins. In the spleen, necrosis in lymphoid follicles, depletion of lymphoid tissues and hypertrophied mesothelium were observed. Tachyzoites in both liver and spleen were rarely seen. In addition, multifocal nonsuppurative encephalitis, severe necrotizing enteritis in the duodenum, pancreatitis and adrenalitis admixed

with many intralesional tachyzoites were noted in other sections. No significant microscopic lesions were detected in the myocardium and kidney.

Histochemistry Results: Many Giemsa-positive tachyzoites were found within and free macrophages throughout the lesions in the affected tissues.

Immunocytochemistry Results: Immunocytochemistry with rabbit anti-*Toxoplasma gondii* antibody showed weakly positive staining in the tachyzoites scattered in the necrotic areas of the involved tissues.

Electron Microscopic Findings: Ultrastructural examination revealed the delicate structures of tachyzoites free and within macrophages. The organisms engulfed in the cytoplasm of infected cells were usually in clusters with occasional dividing by endodyogeny. The elongate tachyzoite is surrounded by a pellicle composed of two membranes and has a truncated cone-shape structure at the anterior end. Nucleus, mitochondria, numerous micronemes, and rhoptries were often observed in the cytoplasm (Figure 4) .

Discussion: Toxoplasmosis is a systemic protozoal infection of humans and many other warm-blooded animals caused by an intracellular coccidian parasite, *Toxoplasma gondii*. Cats (and other felines) are the only animals that can excrete oocysts in their feces and are important in the transmission of *Toxoplasma gondii* to other animals. Three pathways by which it is mainly spread are transplacental transmission, ingestion of infected tissues, and ingestion of food or water contaminated with sporulated oocyst from cat feces. Most common in humans, the congenital form of toxoplasmosis is often associated with severe encephalitis with cerebral calcification, hydrocephalus, microcephaly, and chorioretinitis. In domestic animals, toxoplasmosis is important in sheep and goat, in which abortion and perinatal deaths can produce economic losses. In acute infection, the ingested sporozoites or bradyzoites in the intestine penetrate the mucosa, change into a fast-multiplying tachyzoites, spread through lymphatics to regional lymph nodes, result in parasitemia, and then in localization and multiplication of the organisms in the body. Systemic toxoplasmosis most often affects the lungs, brain, lymph nodes, liver, pancreas, intestinal muscularis, myocardium, uterus, and placenta. Pulmonary lesions are probably most consistently observed. The lesions in the varying organs are morphologically similar in most animal species, but varying degree in severity. They are characterized by multifocal coagulation necrosis and mononuclear inflammatory cell reaction associated with the finding of tachyzoites.

In the Department of Pathobiology, Pig Research Institute, Taiwan (PRIT), histopathological diagnosis of toxoplasmosis (1985-1994) has been reported in 11 species including pig, dog, rabbit, pheasant, tiger, red-handed tamarin, red kangaroo, red necked wallaby, lion, and squirrel. The organisms most often affect the lungs, liver, brain, myocardium, lymph nodes, intestine, pancreas, adrenal gland, and spleen. The lesions are similar to those described previously.

Toxoplasmosis is one of the most important zoonosis. Serologic surveys indicate that the infection is widespread in humans and domestic animals. An estimated 30-40% of adult human beings, 54.3% of health cats, and 1 to 69% of swine examined in the USA may have antibodies to Toxoplasma gondii. Ingestion of cysts in undercooked or uncooked meat is most likely an important source of human transmission. Both humoral and cellular immunity develop following infection, but cellular response appears to be critical and significant. This may reduce the severity of infection. In absence of a cell-mediated immune reaction, treatment with immunosuppressive drugs (e. g. organ transplantation), and other factors depressing immunity (e. g. AIDS), a chronic infection may become reactivated and proliferation of tachyzoites often occurs in spite of the presence of antibody. The mechanism of entry of tachyzoites into host cells leading to tissue necrosis has been attributed to their specific anterior conoid structure which has a screw-like function to penetrate the host cell membrane. Additionally, cytoplasmic rhoptries contain surface-active substances and penetration-enhancing factor to cause membrane expansion, invagination and destruction.

Diagnostic Criteria:

1. Histologic identification: tachyzoites in tissue sections or smear of body fluids demonstrated with H&E stain and Giemsa's stain.
2. Electron microscopy: multilayered pellicle, a conoid, rhoptries, micronemes, a nucleus, and other organells.
3. Immunohistochemistry: immunofluorescence and immunoperoxidase.
4. Parasite isolation: blood, body fluids, and aborted tissues.
5. Serologic tests: Sabin-Feldman dye test, indirect hemagglutination, indirect fluorescence, and ELISA tests.

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Comparative Pathology Case 17

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Clinical History: Sow, was presented with signs of fever, dyspnea, and abortion (Figure 1) .

Diagnosis: Severe, diffuse interstitial pneumonia, multiple focal necrosis, toxoplasmosis.

Gross Findings: The entire lung of this sow was swollen, firm, wet, and heavy, with many tiny white spots of necrosis scattered throughout the parenchyma (Figure 2) . The bronchial lymph node and hepatic lymph node were characteristically moist and swollen.

Histopathological Findings: The slide section of lung showed marked alveolar lining cell proliferation. The blood vessels were severely congested and the alveolar lumen filled with hyaline edematous fluid and many macrophages. The macrophages originated from infiltration or proliferation usually containing tachyzoites inside their cytoplasm; multifocal necrotic foci were distributed in the parenchymatous tissue (Figure 3) .

Immunocytochemical Results: Positive reaction was seen in the proliferation of macrophages with ABC method.

Discussion: Toxoplasmosis is caused by infection with Toxoplasma gondii. The pathologic changes are associated with necrosis of host tissue caused by the tachyzoites. T. gondii often induced multifocal necrosis but usually absence of apparent cellular reactions in the variety of organs. The pathogenesis of necrosis is still not clear yet. It might be in part due to the property of intracellular parasitism of some kinds of secretory antigens or exotoxins.