Analysis of Nucleoprotein Gene of Influenza A Viruses Isolated from Human, Swine and Avian Species in Thailand

N. Thippamom1, P. Kittikoon1, R. Thanawongnuwech1, D. Sreta1, Y. Poovorawan2, K. Suwannakarn2, S. Damrongwatanapokin3, A. Amonsin1*

1Faculty of Veterinary Science, Chulalongkorn University, Bangkok, 10330, Thailand  2Center of Excellence in Clinical Virology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand  3USAID/ Regional Development Mission-Asia, Bangkok, Thailand  *Corresponding author: Alongkorn.A@chula.ac.th

Keywords: avian, human, influenza A, nucleoprotein, swine

Introduction

Influenza A virus caused a serious threat to public health world wide, particularly the virus circulating in human and animals such as swine, bird and horse. The virus genome contains 8 segments of single strand RNA that encode 10-11 proteins (8). Among those genes, NP gene plays a major role in host range or host species barriers for influenza A virus (6). At least two large classes of NP gene had been determined by phylogenetic analysis containing human and nonhuman classes (1). Recently, novel pandemic influenza virus (H1N1, 2009) emerged and spread worldwide that containing genes from human, swine and avian viruses. However, some certain influenza A isolates were shown to have NP gene that might not be host specific, such as the swine origin influenza 2009 virus (S-OIV) in human (4). NP genes of S-OIV were suggested to originate from the classical swine influenza virus. The purpose of this study is to determine the genetic variation of the NP gene of influenza viruses isolated from human swine and avian in Thailand.

Materials and Methods

Viral RNA was extracted from allantoic fluid by using a QIAmp viral RNA mini kit (Qiagen, GmbH, Germany). This was followed by reverse transcription and amplification of the NP gene by polymerase chain reaction (PCR) with specific primers according to Hoffmann et al., 2001 with some modifications (3). The PCR products were separated by using 1.5% agarose gel electrophoresis and were purified by the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany). DNA sequencing was carried out by dideoxynucleotide chain termination technique. Sequences were edited by using Chromas version 1.45 (Technelysium Pty. Ltd., Australia) and the Bioedit version 7.0.0 (2). Finally, sequences were aligned using the MegAlign program (DNASTAR, Madison, WI). Phylogenetic trees analyses were conducted in MEGA version 4 (7) using neighbor-joining method with 1000 times bootstrapping replicates.

Result and Discussion

Phylogenetic analysis of 49 different NP nucleotide sequence of human (n=18), swine (n=16) and avian (n=15) Thai isolates is presented in Fig 1. Two major groups are found, one containing viruses from human lineages, the other from avian and swine (Classical and European swine) lineages. In the previous report, the swine lineages were branching with the human lineage by phylogenetic analysis (5). But in this study, the swine lineages of Thailand were branching with avian lineage. Obviously, it was indicated that NP gene of human, swine and avian viruses in Thailand are highly conserved with host specific.

Comparison of amino acid sequence of six positions (position 16, 33, 100, 136, 283 and 293) recognized to have host specificity of the Influenza A viruses was performed. It was found that all six positions in human and avian lineages were highly conserved. As for swine lineage, variation was observed in position 33, 100 and 136. Most isolates (12 out of 16 swine isolates) showed similarity with avian lineage (Table 1.) On the other hand, our swine isolates which similarity to human isolates include sw/Thailand/NIAH586-4/05(H3N2), sw/Chachoengsao/NIAH586/05(H3N2) and sw/Ratchaburi/NIAH874/05 (H3N2); these four isolates belong to classical swine lineage. Four swine isolates are different from avian lineage as shown on Table 2.
Acknowledgements

This study was supported by the Thailand Research Fund (TRF Master Research Grants: TRF-MAG window II 2008, MAG-WII515S055) and Chulalongkorn University Fund (Ratchadaphiseksompoht Endowment Fund).

Table 1  Nucleoprotein phenotypic markers; six conserved amino acids determinants among human, swine and avian influenza viruses

<table>
<thead>
<tr>
<th>Amino acid position</th>
<th>Amino acid in indicated strain:</th>
<th>Human</th>
<th>Swine</th>
<th>Avian</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td></td>
<td>D</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>33</td>
<td></td>
<td>I</td>
<td>V/I</td>
<td>V</td>
</tr>
<tr>
<td>100</td>
<td></td>
<td>V</td>
<td>R/V</td>
<td>R</td>
</tr>
<tr>
<td>136</td>
<td></td>
<td>I</td>
<td>L/I</td>
<td>L</td>
</tr>
<tr>
<td>283</td>
<td></td>
<td>P</td>
<td>L</td>
<td>L</td>
</tr>
<tr>
<td>293</td>
<td></td>
<td>K</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>

Table 2  Variation of amino acid positions of four swine isolates similar to human than avian lineage

<table>
<thead>
<tr>
<th>Virus</th>
<th>Amino acid position</th>
<th>Amino acid</th>
<th>position</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>33°</td>
<td>V</td>
<td>1</td>
</tr>
<tr>
<td>Avian</td>
<td>100°</td>
<td>R</td>
<td>L</td>
</tr>
<tr>
<td>Sw/NIAH586-4/05(H3N2)</td>
<td>136°</td>
<td>I</td>
<td>V</td>
</tr>
<tr>
<td>Sw/NIAH586/05(H3N2)</td>
<td></td>
<td>I</td>
<td>V</td>
</tr>
<tr>
<td>Sw/NIAH-03/03(H3N2)</td>
<td></td>
<td>I</td>
<td>V</td>
</tr>
<tr>
<td>Sw/NIAH874/05(H3N2)</td>
<td></td>
<td>I</td>
<td>V</td>
</tr>
</tbody>
</table>

*Most of swine isolates similar to avian lineage, excluding four isolates similar to human lineage

Fig.1  A phylogenetic tree of 49 NP genes of influenza A viruses isolated from human, swine and avian species in Thailand

References

5. Reid et al., 2004. J. Virol. 78: 12462-12470
Food Frequency Questionnaire in Cancer Animals in Bangkok Metropolitan

N. Thongsoi1, P. Teewasutrakul2, A. Rungsipipat1,2*

1Department of Pathology, 2Oncology Clinic, Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand  *Corresponding author: anudep.r@chula.ac.th

Keywords: Bangkok, cancer animals, food frequency questionnaire

Introduction
An incidence of cancer animals presented at oncology clinic, Small Animal Hospital, Chulalongkorn University is increasing every year as in humans. The cause of cancer is a major scope of interest. At present, many scientists have proposed a hypothesis that exposure to environmental carcinogen, including many xenochemicals, may account for the recent growing incidence of cancer, as there is evidence that the environment has changed over the time period preceding this figure (1). The two major contributing environmental factors in concern are smoking and diet (2). As a result, the extent to which diet is capable of causing somatic alteration in genes known to be involved in the causation of cancer, or is able to prevent or mitigate these alterations, is an emerging area of research (3). In Thai Veterinary practice, the relation of dietary factor and cancer has not been conducted yet. For these reasons, the main objective of this study was to investigate the baseline data of the overall pictures of foods given in cancer animals. We proposed that all animals including dogs and cats have their specific foods for each species. If the animals are fed with foods that are modified from their native foods, this will lead to health problems or even certain diseases including cancer. Some types of foods such as seasoned grilled meat, milk and dairy products, desserts, dog sticks and human snacks are considered extraordinary foods in our purpose as they are not the food in the natural way of living for animals. We found a high percentage of these kinds of foods fed in cancer animals.

Materials and Methods
We developed a food frequency questionnaire for cancer animals presented at the Small Animal Teaching Hospital, Chulalongkorn University during August 2008-2009 and 20% of the owners of the prevalent cases were interviewed with one interviewer (250 cases). The cancer types were classified into seven groups according to their cell origins (Table 1). The food items of extraordinary foods were grouped into seasoned grilled meat, milk and dairy products, desserts, dog sticks and human snacks. The results shown were a part of all the results obtained.

Results and Discussion
Regarding with diets suspicious of association with cancer, our proposed diets are in major of concerns. In the past decade, there have been extensive studies in humans revealed the association between these kinds of foods and some cancers. For example, there is strong evidence that grilled meat generated potent carcinogens, heterocyclic amines (HA) that causes cancer in experimental animals and were regarded as the list of substances reasonably anticipated to be human carcinogens (4, 5). In addition, greater intake of milk or dairy products has been consistently associated with an elevated risk of prostate, breast, colorectal and lung cancer in several studies (6). Recently, it has also been shown that high consumption of sugar and high sugar foods may be associated with pancreatic cancer risk (7, 8). In regarding with dog sticks and human snacks, food additives such as, preservatives, food coloring and food seasonings were considered. Many additives were proven that high doses have caused cancer in laboratory animals and some food additives are considered to be possibly carcinogenic to humans (9). As increased incidences of cancer also occurred in pets, this raises questions about whether lifestyle and dietary factors may influence disease risk as they do in humans. Our results demonstrated the apparently high percentage of more than 50% of cancer animals were fed with seasoned grilled meat, desserts, milk and dairy products, a lesser extent were found in dog sticks and human snacks (Table 1). Interestingly, we found that most cancer animals are regularly fed the repetitive foods including these extraordinary foods for almost the whole of their lives. Because these foods are not thoroughly studied whether this prolonged feeding in pets may have undesired effects including carcinogenesis, awareness should be concerned and case-control studies should be performed in order to clarify this association. For the results obtained, the overall percentage went toward the trend of risk as described in human. Dietary factor might be one of the important factors contributing to increased incidences of cancer in pets at the present time. The association between these kinds of foods and cancer in this study is being evaluated.

Table 1 Percentage of some extraordinary foods

<table>
<thead>
<tr>
<th>Cancer types</th>
<th>Seasoned grilled meat</th>
<th>Milk &amp; dairy products</th>
<th>Desserts</th>
<th>Dog sticks</th>
<th>Human snacks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mast cell tumor (n=76)</td>
<td>92.11</td>
<td>73.68</td>
<td>86.84</td>
<td>56.58</td>
<td>53.95</td>
</tr>
<tr>
<td>Malignant mesenchymal tumor (n=46)</td>
<td>71.74</td>
<td>65.22</td>
<td>63.04</td>
<td>58.70</td>
<td>41.30</td>
</tr>
<tr>
<td>Lymphoid tumor (n=37)</td>
<td>83.78</td>
<td>75.68</td>
<td>89.19</td>
<td>67.57</td>
<td>45.95</td>
</tr>
<tr>
<td>Malignant epithelial tumor (n=33)</td>
<td>75.76</td>
<td>69.70</td>
<td>78.79</td>
<td>36.36</td>
<td>54.55</td>
</tr>
<tr>
<td>Mammary tumor (n=25)</td>
<td>80.80</td>
<td>89.92</td>
<td>82.61</td>
<td>39.13</td>
<td>78.26</td>
</tr>
<tr>
<td>Others (n=10)</td>
<td>90</td>
<td>70</td>
<td>70</td>
<td>50</td>
<td>60</td>
</tr>
</tbody>
</table>

References
Detection of Porcine Circovirus Type 2 Antibodies in Gilts Culled due to Reproductive Failure

P. Tummaruk1*, S. Kesdangsakonwut2, R. Tantilertcharoen3

1Department of Obstetrics, Gynaecology and Reproduction; 2Department of Pathology; 3Veterinary Diagnostic Laboratory, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand 10330

*Corresponding author: Padet.T@chula.ac.th

Keywords: gilts, porcine circovirus type 2, reproductive failure

Introduction
Porcine circovirus (PCV) is a small, non-enveloped, single-stranded DNA virus with a circular genome (1). The virus has been recognized since 1974 (2). In late 1990s, a new emerging disease in pigs so call ‘post’ weaning multisystemic wasting syndrome (PMWS) was reported to be associated with PCV (3). In general, the pathogenic PMWS-associated PCV is designating as porcine circovirus type 2 (PCV2) and the nonpathogenic PCV as porcine circovirus type 1 (PCV1) (4). In pregnant gilts and sows, transplacental infection has been demonstrated (4, 5). Recently, reproductive failure after PCV2 infection via artificial insemination has also been demonstrated (6). The first evidence of PCV2 infection in Thailand has been reported since 1999 in 7-8 weeks old pigs (7). However, the association between PCV2 infection and reproductive failure has never been reported. The objective of the present study was to determined sero-prevalence of PCV2 in replacement gilts culled due to reproductive failure in association with infection of some other common reproductive diseases in Thailand, i.e., Aujeszky’s disease (AD), porcine parvo virus (PPV) and porcine reproductive and respiratory syndrome (PRRS).

Materials and Methods

Animal: Twenty-seven gilts culled due to reproductive failure from 5 swine commercial herds in Thailand during August 2005 to July 2006 were included. Historical data including the gilt’s identities, date of birth, date of entry into the herd, date of first observed oestrus, date of first insemination, date of culling and culling reasons were collected from the herds. In most cases, the gilts were vaccinated against foot-and-mouth disease, swine fever, AD and PPV at 22-30 wk of age. None of the gilts were vaccinated against PCV2. All herds were breeding herd and the sows on production numbering between 900 to 3,500 sows/herd. The gilts entered the gilt pools at 22 to 24 wk of age. In the gilt pools, water was provided to ad libitum from water nipples. The feed (a corn-soybean-fish base, 16-18% CP, 3,000-3,400 kcal/kg ME, 0.85-1% lysine) were provided about 3 kg/day.

Blood collection: Blood sample were collected from the gilts prior to slaughter and were sent to the laboratory within 24 h of culling. Serum was obtained and stored at -20°C until assays.

Detection of porcine circovirus antibody: Antibodies titers against PCV2 were detected using SERELISA® PCV2 Ab Mono Blocking (Synbiotics Europe SAS, Lyon, Cedex 07, France). The procedure was carried out according to the manufacturer’s instruction. Briefly, the controls and samples are placed in wells sensitized with anti-PCV2 antibodies bound specifically to purified PCV antigen. After a wash step to eliminate the non-associated fractions, an ati-PCV2/peroxidase conjugate is added. If there is no specific anti-PCV2 antibody in the sample, the anti-PCV2/peroxidase conjugate is free to attach forming an antigen-antibody-conjugate complex. After second wash step, the coupled enzyme conjugate is revealed by the addition of substrated, which transforms into color product. Optical density (OD) was measured at 450 nm. The OD are recorded and used to determine the presence or absence of the antibodies as a function of the threshold values. The ratio between sample OD and negative control OD was calculated. If S/N ratio was ≥0.15, the sample was defined as ‘positive’ and if the S/N ratio was ≥0.2, the sample was defined as ‘negative’ and if S/N ratio was between 0.15 and 0.2, the sample was defined as ‘suspected’.

Detection of AD, PPV and PRRS: Antibodies against PPV were determined using haemagglutination inhibition
HI test. Gilts were considered to have low antibody levels when HI titers were <1:512 and titer ≥1:512 were considered high. Antibody of PRRS virus was determined using HerdChek® PRRS virus antibody test kit 2XR (IDEXX Lab., Inc., USA). Antibody against G1 antibody of AD virus was determined using HerdChek® Anti-PRV gpl test kit (IDEXX Lab., Inc., USA).

Statistical analyses: The statistical analyses was performed by SAS (SAS version 9.0, Cary, NC, USA). Frequency analysis was carried by using FREQ procedure. p<0.05 were considered to have statistical significant.

Results and Discussion

The gilts were culled at 300.3±37.5 d of age. The reasons for culling included anestrus (22 gilts), repeat breeding (3 gilts) and vaginal discharge (2 gilts). Of the 3 positive PCV2 gilts, 2 gilts were culled due to anestrus and one gilt was culled due to vaginal discharge. All of the PCV2-positive gilts (3/3, 100%) had high PPV titer (>1:512), while 7/9 (77.7%) PCV2-negative gilts and 9/15 (60%) PCV2-suspected gilts had high PPV titer (p=0.32). PRRS sero-positive gilt was found in 2 out of 3 PCV2-positive gilts. One out of the three PCV2-positive gilts was also co-infected with AD.

The present study indicated that PCV2 infection occurred in replacement gilts. In all of the positive PCV2 cases, the gilts are likely to be co-infected with PPV. Co-infection between PPV and PCV2 is common and have been reported as a cause of severe PMWS in nursery pigs (8, 9). Based on the influences of PPV and PCV2 co-infection on PMWS in nursery pig, this combination might also cause reproductive failure in the gilts. In addition, the present study also demonstrated that co-infection between PCV2 and PRRS (2/3 gilts) and/or AD (1/3 gilts) might possibly occurred under field conditions. These co-infections might increase the severity of the clinical signs after PCV2 infection (4). In the present study, PCV2 positive gilts were culled due to anestrus (2/3) and vaginal discharge (1/3). Base on these field data, it could not be roll out that PCV associated disease causes these reproductive failures since other management problems might also involve. However, awareness on the PCV associated disease in replacement gilts should be raised.

Acknowledgements

The present study was funded by the Faculty of Veterinary Science, Chulalongkorn University (RG12/2552).

References

Study of Aquaporin 1 (AQP1) on Acute kidney injury

W. Prachasilchai¹*, K. Ito², M. Ikeda²

¹Small Animal Clinic, Department of Companion Animals and Wildlifes, Faculty of Veterinary Medicine, Chiangmai University, Chiangmai 50100, Thailand
²Department of Veterinary Pharmacology, Faculty of Agriculture, University of Miyazaki, Miyazaki 889-2192, Japan *Corresponding author: w.pracha@chiangmai.ac.th

Keywords: acute kidney injury, aquaporin 1, mice, renal ischemia-reperfusion injury, western blotting

Introduction

Acute kidney injury (AKI), also known as acute kidney failure or acute renal failure is a syndrome associated with a high mortality rate of up to 50%. AKI is characterized by rapid decline in glomerular filtration rate and is a major cause of in-hospital morbidity and mortality. Renal ischemia-reperfusion injury (IR) is an important cause of AKI (1). Renal IR model is the most widely used as an experimental model to investigate the pathophysiology of AKI (2). Recently, many studies have revealed that protein water channels, referred to as aquaporins (AQPs) are involved in increasing the osmotic permeability of membranes. The transepithelial movement of water through urinary system is an essential process for body metabolism. AQP1 is expressed in epithelial and endothelial cells in several tissues (3). This study has examined the expression of AQP1 in IR mice.

Materials and Methods

The renal ischemia-reperfusion procedure was performed (2). Male ddY mice (7 weeks old) were anesthetized by pentobarbital (65 to 75 mg/kg) and operated abdomen. A microvascular clamp (Roboz, MD) was placed on each renal pedicle for 35 min. After the ischemic period, the clamps were removed. For renal function analyses, 50 µl blood samples were collected from tail blood vessel by a hematocrit capillary under ether anesthesia. Plasma urea nitrogen and creatinine levels were measured by an autoanalyzer (Fujidrichem®, Japan). Preparation of kidney extracts and western blot analysis were performed (2). After separation by SDS-PAGE, the protein was transferred onto a polyvinylidene difluoride membrane and analyzed by immunoblotting. Antibody used anti-AQP1 antibodies (Santa Cruz Biotech., Inc., CA). Statistical comparisons among group mean values were performed by Student’s t-test.

Results and Discussion

Samples of mice’s kidney were investigated by Western blotting procedures. AQP1 was up-regulated at 24 h after IR. At 72 h after IR, AQP1 was down-regulated significantly (p<0.05). These data correlated with plasma urea nitrogen and creatinine concentrations (2).

The present study demonstrated that AQP1 expression is associated with IR mice. Therefore, it is possible that AQP1 is involved in AKI. It might be concluded that AQP1 is may be used as a marker for AKI.

Acknowledgements

We acknowledge members of Veterinary Pharmacology, University of Miyazaki.

References

Biliary Changes with the Development of *Opisthorchis viverrini*-Associated CCA in a Hamster Model

S. Tangkawattana¹,⁴,⁵*, P. Tangkawattana², B. Sripa³,⁴,⁵

¹Department of Veterinary Pathobiology, ²Department of Veterinary Anatomy, Faculty of Veterinary Medicine, ³Department of Pathology Faculty of Medicine, ⁴Tropical Disease Research Laboratory, ⁵Liver Fluke and Cholangiocarcinoma Research Center, Khon Kaen University, Khon Kaen 40002, Thailand.

*Corresponding author: sirikach@kku.ac.th

Keywords: biliary, cholangiocarcinoma, hamster, Opisthorchis

Introduction

Since Syrian golden hamster has relatively high resistance to N-nitrosodimethylnitrosamine (NDMA) for hematoma induction (4), it would be the best studying model of NDMA and OV co-operativity in enhancing CCA genesis (2).

Materials and Methods

1) Induced tumor by 50 OV metacercariae (OV group) and/or daily dose of 12.5 ppm of NDMA (OVDMN group) for 8 wks, PO.
2) Collected livers on wks 1-4, 8, 12 & 24 post-infection for histopathology and immunohistopathology of Ki-67 (Clone MM1, Novacastra, UK).

Results and Discussion

Tumor occurred only in OVDMN group (56%) and could be classified into 4 types without statistical significance (Fig 1, Table 1&2). The intratumorous lesions were also shown (Fig 2). Positive Ki-67 staining gradually increased with time. Proliferation was predominant in pre-cancerous & cancerous lesions could be classified into 4 grades (Table 2). Dysplasia should be the result of cell maturation disturbance by NDMA, OV or its excretion/secretion so being considered as pre-cancerous changes (Sripa (2003). Our unpublished data suggested that OV did not inhibit apoptosis but promote cell proliferation in vitro. Therefore, cholangiocarcinogenesis possibly occurred in a sequence of hyperplasia, dysplasia and CCA (Hughes et al., 2005).

Acknowledgements


References

Concurrent Transitional Cell Carcinoma and Leiomyosarcoma in the Urinary Bladder of a Fishing Cat (*Prionailurus viverrinus*)

P. Kongmakee*, A. Sommanustweechai¹, D. Tongthainan², R. M. Bush², Jiraporn Sritun³, C. Kasorndorkbua³

¹Conservation Research and Education Centre, Zoological Park Organization, Bangkok, Thailand  ²Khao Kheow Open Zoo, Zoological Park Organization, Chonburi, Thailand  ³Department of Pathology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand  *Corresponding author: p_jeangy@hotmail.com

**Keywords**: fishing cat, leiomyosarcoma, *Prionailurus viverrinus*, transitional cell carcinoma, urinary bladder

**Introduction**

The Fishing Cat (*Prionailurus viverrinus*) is a medium-sized cat living range spans throughout tropical Asia. Fishing Cat is listed as endangered on the IUCN Red List of Threatened Species and is included in CITES Appendix II. Transitional cell carcinoma (TCC) is the most common urinary bladder tumor in domestic cats and fishing cat (1).

**Materials and Methods**

A five-year-old male fishing cat was examined due to the history of hematuria and anorexia. Blood urea nitrogen (BUN) was >140.0 mg/dl and creatinine was 12.7 mg/dl. A clinical examination was repeated two weeks later because of inappetite, lethargy, stranguria and hematuria. The abdomen was grossly enlarged. An abnormal mass, 1.7 cm. in diameter was found on the urinary bladder wall from abdominal radiograph. Thoracic radiograph showed increased opacity of the lung. The cat was then very depletes, became moribund and died 2 days later.

**Results and Discussion**

At necropsy, bilateral renomegaly with marked dilation of renal pelves, pressure atrophy of adjacent medulla and a large hematoma within the left renal pelvis were evident. A tumorous mass was located at the trigone of urinary bladder (Fig. 1). Microscopic examination revealed neoplastic transitional epithelial cells arranged in a papillary pattern with marked anisokaryotic, hyperchromatic nuclei and a high mitotic index. Adjacent to the carcinoma, there was an invasive but well-circumscribed highly cellular mass characterized by irregular bundles of spindle cells with elongate and blunt-ended single nuclei, typical of leiomyosarcoma. Necrotic foci were scattered throughout both neoplastic areas. Immunohistochemical staining for cytokeratin and vimentin were done to confirm the transitional cell carcinoma and leiomyosarcoma, respectively (Fig. 2b, 3b). The carcinoma cells stained positive in the cytoplasm for cytokeratin, a marker for epithelial cells, and the leiomyosarcomatous cells positive for vimentin in the cytoplasm, indicating a mesenchymal cell origin. TCC is reportedly a common tumor in fishing cats. A high prevalence of TCC in fishing cats is well documented, however the cause is currently unknown (2). The effect of nutrition has been implicated in the pathogenesis of TCC in the species. However leiomyosarcoma in the muscle layer of urinary bladder has not been reported. To the authors’ knowledge, this is the first report of concurrent TCC and leiomyosarcoma in the fishing cat.

**References**

Differential Expression of Putative Canine Distemper Virus Receptors following \textit{in vitro} Infection of Canine Glia

S. Techangamsuwan\textsuperscript{1*}, C. Puff\textsuperscript{2}, K. Wewetzer\textsuperscript{2,3}, W. Baumgärtner\textsuperscript{2,4}

\textsuperscript{1}Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, 10330 Thailand
\textsuperscript{2}Department of Pathology, University of Veterinary Medicine, Hannover, 30559 Germany
\textsuperscript{3}Center of Anatomy, Hannover Medical School, Hannover, 30625 Germany
\textsuperscript{4}Center of Systems Neuroscience, Hannover, Germany

*Corresponding author: somporn62@hotmail.com

Keywords: canine distemper virus, CD9, CD150, glia, p75NTR

Introduction

Canine distemper (CD) is a highly contagious and immuno-suppressive viral disease among all families of the order Carnivora. In contrast to the lesion of peripheral nervous system, which has not been addressed so far, demyelination is the main sequela of canine distemper virus (CDV)-induced lesion in the central nervous system in which its pathogenesis is remained elusive. Currently, the only known morbillivirus receptor is signaling lymphocyte activation molecule (SLAM, CD150) which is expressed on a variety of different lymphoid cell subpopulations (1). CD9, a tetraspan transmembrane protein, plays a role in CDV uptake by target cells, cell-cell syncytium formation and progeny virus production (2). The p75 neurotrophin receptor (p75\textsuperscript{NTR})-positive brain cells has been recently shown as a preferential infected cells following CDV inoculation \textit{in vitro} (3). In the present study, we investigated the expression of these molecules in CDV-infected canine glial culture.

Materials and Methods

Cultures of olfactory ensheathing cells (OECs) and Schwann cells (SCs) obtained from 2 Beagles (6-month-old) were isolated, purified and maintained under standard conditions (37°C, 5% CO\textsubscript{2}, water-saturated atmosphere) (4). Cells from passages 5-7 were seeded in 6-well PLL-coated plates (Nunc\textsuperscript{TM}) (1.6x10\textsuperscript{5}cells/well) and inoculated with CDV Onderstepoort strain (CDV-Ond) at a multiplicity of infection (MOI) of 0.1. Samples were collected following 2 h (hpi), 3 and 10 days post infection (dpi) for quantitative real-time PCR analysis (Mx3005PTM QPCR System, Stratagene\textsuperscript{\textregistered} Europe, Amsterdam, the Netherlands).

The sequence of primer pairs were used as follows:

\textbf{ICDV-N}  
F: 5'-GCT CTT GGG TTG CAT GAG TT-3' (83 bp)  
R: 5'-GCT GTT TCA CCC ATC TGT TG-3'  

\textbf{SLAM}  
F: 5'-TGG AAA GCA GGA GGG AAA ATG A-3' (280 bp)  
R: 5'-TGA GGG CCG AGG CTG AGG TG-3'  

\textbf{CD9}  
F: 5'-TTT GGC TTC CTC TTG GTG AT-3' (227 bp)  
R: 5'-GGG CAG ATG TCG GAG ATA AA-3'  

\textbf{p75\textsuperscript{NTR}}  
F: 5'-TGA GTG CTG CAA AGC CTG CAA-3' (229 bp)  
R: 5'-TCT CAT CCT GGT AGT AGC CGT-3' (6)

Data were normalized with a normalization factor achieved by geometric averaging of the two most stable housekeeping genes (EF1, HPRT) using the geNorm software version 3.5.

Results and Discussion

In controls, CD9 and p75\textsuperscript{NTR} were significantly expressed in SC comparing to OEC (*) while SLAM was detected in a low copy number only in OEC at 2 hr after seeding. Following CDV-Ond infection, the expressions of CD9 and p75\textsuperscript{NTR} were not differed from the non-infected controls, however the higher copy numbers remained confined to SC (*). Interestingly, this is the first demonstration that canine SC up-regulated the expression of SLAM after early CDV infection \textit{in vitro}. In addition, the differential expression of CD9 and p75\textsuperscript{NTR} indicated the significant difference between both closely-related glial cell types at a molecular level which is in agreement with the previous studies (8).

Acknowledgements

This work is financially supported by the Deutsche Forschungsgemeinschaft (DFG, Germany) grant BA 815/9-1 and partly funded by Grants for Development of New Faculty Staff (GDNS 52-089-31-007)

References

Pancreatic Islet Amyloidosis with Signs of Diabetic Ketoacidosis in a DSH Cat: A Clinicopathological Model for Human T2DM

T. Mamom1*, S. Pavarutipaisit2

1Department of Pathology, Faculty of Veterinary Medicine  2Histopathology Section, Mahanakorn Veterinary Diagnostic Center (MVDC), Mahanakorn University of Technology  *Corresponding author: thanonsa@mut.ac.th

Keywords: amyloidosis, Congo red, diabetes, histopathology, ketoacidosis, pancreatic islet

Introduction

Spontaneous feline diabetes mellitus (FDM) is close resemblance to human type 2 diabetes mellitus (T2DM) clinically and pathologically. Clinical similarity between FDM and T2DM include clinical onset of middle age (between 9 and 13 years of age in cat), obesity and resistance to ketoacidosis. The most striking similarity is pathological finding of pancreatic islet amyloidosis (IA) and partial loss of β-cells (1, 2). Islet amyloidosis was reported in more than 90% of feline diabetes cases. It can also occur in human and macaques but not in rats and mice. More are known about islet amyloid polypeptide (IAPP) or amylin, precursor protein found in β-cells secretory vesicle. Amylin is co-secreted with insulin, therefore it is usually found in association with diabetic syndrome especially in aged animal (3). Increase amylin secretion predisposes IA. At present, several studies supported the role of IA in development of FDM and T2DM. Many data support the concept that amyloid fibril derived from amylin are cytotoxic and associated with apoptotic cell death and/or necrosis. Human is resistant to diabetic ketoacidosis (DKA). In cat, DKA is characterized by hyperglycemia, hyperketonemia and metabolic acidosis. It is a serious complication of DM and requires emergency treatment. Other complications are hypoglycemia, hypokalemia, dehydration and hyperosmolarity (4).

The aim of this study is to demonstrate the incidence of pancreatic islet amyloidosis in association with diabetic ketoacidosis (DKA), a serious complication of FDM, in a DSH cat as animal model for human type 2 diabetes mellitus (T2DM).

Materials and Methods

Case History: A 20-year-old castrated male domestic short hair (DSH) cat with clinical sign of depression, anorexia and weight loss without detectable signs of PU/PD was submitted to a private small animal clinic in Bangkok. Physical examination and complete blood count were unremarkable. Serum biochemistry profile revealed 10 fold increase in ALT while BUN and creatinin value was still in normal range. Moderate to severe hepatocellular injury was diagnosed. Various kinds of supportive treatment were administered and ALT level was thereafter declined to a level of mild hepatocellular damage. The cat died few days after voiding of consecutive treatment. The carcass was submitted to MVDC for investigation. Necropsy was performed and tissue samples were collected and sent to histopathology laboratory.

Results and Discussion

Necropsy result: The carcass was in fair nutritional state but revealed large amount of fat deposition in abdominal and thoracic cavities. The liver was pale and friable. The kidney was pale and some petechial hemorrhages were seen at corticomedullary junction. Multiple white foci were detected at pancreatic surface. Pulmonary alveolar emphysema and atelectasis were found. Multiple gastric ulcers with subsequent melena were observed. Other organs showed no remarkable lesion. Using urine strip analysis, marked glucosuria was detected and ketonemia was found in abdominal fluid.

Histopathological result: Severe diffuse panlobular fatty changes with stenosis of adjacent sinusoids were observed. Fatty changes were also seen in myocardial fiber. Multinodular hyperplasia of pancreatic acinar cells with accumulation of many neutrophils was observed. Accumulation of pink homogenous material at pancreatic...
islets and vacuolization of endocrine cells in islets were detected. This material stained deep orange with Congo red and exhibited apple green color using polarized light microscope.

**Morphological diagnosis:** Hepatic lipidosis as a result of diabetic ketoacidosis associated with pancreatic islet amyloidosis was diagnosed.

**Discussion:** Feline diabetes mellitus is commonly observed in aged animal. In this case, FDM was undiagnosed clinically probably due to lack of the history of PU/PD. Hepatocellular injury with increased ALT in this animal is due to hepatic lipidosis from abnormal carbohydrate or fat metabolism. Islet amyloid deposition may lead to decrease number or function of $\beta$-cells to secrete sufficient insulin. Delayed or undiagnosed DM results to a serious complication of diabetic ketoacidosis (DKA) as observed in this animal. The animal may die from metabolic acidosis and hypokalemia. Lipolysis caused increase fatty acid into the liver and activation of liver enzymes to metabolize fat and form ketones, acetoacetate and $\beta$-hydroxybutyrate. These metabolic products cause acidosis in affected animal. Pancreatitis was reported as a concurrent finding in human with T2DM. It might be the result of marked hyperlipidemia. The cat in this study was also experienced pancreatitis. Obesity has been reported as a risk factor of FDM and human T2DM. The pattern of fat deposition is important. Fat deposition in abdomen, called central obesity, increases the risk of diabetes significantly in comparison with peripheral obesity (5).

**Acknowledgment**

The author thanks Mahanakorn University of Technology (MUT) for financial support. Special thanks were given to Dr. Chotiga for given animal clinical information and Ms. Siriwan, staff of MVDC, for all technical supports.

**References**


Fig. 1-8 Gross, histopathology and special stains:  
Fig. 1 Marked fat deposition (*) in visceral organs, Li: liver, Sp: spleen.  
Fig. 2 Pale yellowish liver with diffuse multifocal telangiectasia (arrow head).  
Fig. 3 Multiple white foci at pancreas (arrow head).  
Fig. 4 Severe diffuse hepatic fatty changes, H&E staining.  
Fig. 5 Tremendous fat droplets in cytoplasm of hepatocytes stained red with Oil-red-O, frozen section.  
Fig. 6 Pancreatic islets showing amyloid deposit (*) with vacuolization of $\beta$-cells (arrow head), H&E staining  
Fig. 7 Congo red staining of islet amyloid (*).  
Fig. 8 Apple green color of islet amyloid (*) under polarized light, Congo red staining.
Systemic Spirochidiasis in a Green Sea Turtles (Chelonia mydas)

T. Suyawanish1*, Y. Matura2, N. Chansue2, N. Pirarat1

1Department of Pathology, 2Department of Veterinary medicine, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand  *Corresponding author: nopadonpirarat@hotmail.com

Keywords: granuloma, green sea turtle, spirochid

Introduction

In Thailand, green turtle is found in both the Andaman and South China Sea coasts. They are listed as globally threatened by the World Conservation Union (IUCN) and are protected by International Law (CITES Appendix I) and Thai Law (WARPA 2535). Nowadays, the turtle population gradually decreased such as by entanglement in fishing gear and drowning, consumption and trade turtle eggs, pollution and disease problem. Although diseases of captive sea turtles reflect husbandry conditions, opportunistic bacterial and fungal infections of the integument and respiratory system figure prominently. Parasitic infection is also a problem to both captive and wild green sea turtle. Among parasitic infection, spirochid trematodes are major pathogens of sea turtles and are associated with strandline mortality worldwide (1). They have been reported as vascular system generalists, with a preference for the heart and arterial system of their turtle hosts (2). Lesions have been seen in many species infected in wild turtles, including green turtle which scatter in USA and Australia. Despite their importance to sea turtle health, none of the life cycles are known for any marine spirochid species. Although the Spirochid eggs can be transmitted via fecal shedding, the intermediate hosts of spirochid trematodes were not identified yet. Pathological study of spirochidiasis in green sea turtle has not been documented scientifically in Thailand. Here, we described the histopathological study of systemic spirochidiasis in a green sea turtle accidentally hit by boat striking.

Material and Methods

A wild green turtle have been accidentally hit by the sailing ship and underwent hospitalization at the sea turtles conservation center, Sattahip Navy Base. The green sea turtle showed clinical signs of anorexia, lethargy, pale anemic mucous membrane and eventual death 2 days after undergoing hospitalization. The necropsy was performed at the sea turtles conservation center. The spleen, liver, lung, intestine and kidney were fixed in 10% neutral buffered formalin for histopathological examination according to standard histopathological procedures.

Results and Discussion

The gross pathology revealed severe traumatic wound at the dorsal carapace surface. The carcass was pale in color. The coelomic cavity was totally filled with bloody content. Severe congestion was observed in the internal organs including spleen, liver and kidney. Severe hypovolemic shock was the cause of death. The histopathology of the section showed Spirochid egg granulomas in every organ examined, however the largest egg granulomas and the highest density of egg granulomas were observed in the spleen. The eggs are golden brown and variably shaped (round, ovoid, or fusiform, sometimes with hooked terminal processes). The brown pigment presented in the vascular thrombi might be an iron-porphyrin compound produced by the spirochids, as this is one of common features of many trematode infections (1). Some granulomatous lesions consisted of well defined thick capsule with fibroblast proliferation. Scattered hemosiderin and melanomacrophages were adjacent to the hemorrhages. Congestion was also observed. The binuclei and macronuclei were present. The focal pyknotic nuclei associated apoptosis were also present. Severe thrombosis and recanalization of splenic blood vessels were noted. The dissemination of Spirochid eggs to the lung in association with granulomatous pneumonia might have caused a problem of oxygen storage during diving and a progressive loss of buoyancy control (4), resulting in traumatic death from a boat strike.

Acknowledgment

The authors would like to thank sea turtle conservation center, Sattahip Navy Base for necropsy assistance.

References

Immunohistochemical Identification of Chytridiomycosis in Poison Dart Frogs (*Dendrobates tinctorius*) in Thailand

N. Pirarat¹*, A. Sommanustweechai², A. Sailasuta¹, S. Kamolnorranart², Y. Une³, B. Siriaroonrat²

¹Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand
²Conservation Research and Education Centre, Zoological Park Organization, Thailand
³Laboratory of Veterinary Pathology, School of Veterinary Medicine, Azabu University, Japan

*Corresponding author: nopadonpirarat@hotmail.com

Keywords: *Batrachochytrium dendrobatidis*, Chytridiomycosis, frog, immunohistochemistry

Introduction

Chytridiomycosis is an emerging disease in amphibians caused by a chytrid fungus, *Batrachochytrium dendrobatidis* (1). It has been killing massive frog populations worldwide since 1996. Until now, at least 1 family and 120 species of amphibians were extinct. The exact killing mechanism has not been clearly elucidated. The infection has been reported broadly in America, Australia, Europe and recently in Japan (3, 4). This disease has never been reported in Thailand. Presumptive diagnosis of chytridiomycosis can be made by the routine H&E staining of affected skin or special fungal stains such as Gomeri Methanamine Silver stain (GMS). However, definitive identification is rather difficult to diagnose based only on routine staining (2). This study reported and characterized chytridiomycosis in captive poison dart frogs in Thailand using immunohistochemistry.

Materials and Methods

Three poison dart frogs (*Dendrobates tinctorius*) received from private market were under quarantine area in the zoo before exhibiting. The frogs eventually died within 3 weeks without any abnormal clinical signs. Three skin sites; gular, abdomen and pelvic patch areas were collected for histopathological examination. The tissue was stained with H&E, PAS and GMS. For Immunohistochemical analysis, primary polyclonal anti-chytrid antibody (1: 250, Dr. Une, Japan) and the secondary antibody conjugated universal immuno-enzyme polymer using Histofine MAX PO kit (Nichirei, Japan) were applied.

Results and Discussion

There are sloughed skins at toe and abdomen in all frogs. No any other systemic signs were seen. Epidermis of a poison dart frog was shown multiple chytrid zoosporangia containing zoospores within the keratin layers of the stratum corneum at abdominal area (Fig.1). A flask shaped zoosporangia with septate thalli which is the characteristic of this disease (3) was also seen. In cross section, the chytrid sporangia can often appear as empty spaces or dark circles. Vacular change of the epidermis accompanied with ballooning degeneration was observed. Ulcerative dermatitis and hyperkeratosis of epidermis were not clearly evidenced. Scattered lymphocytic infiltration in the epidermis was found. Immunohistochemistry, the fungal thalli were clearly visible and appeared as sac-like bodies in the stratum corneum, corresponding to the morphology seen with the routine histological stain. According to climate change issue, amphibian populations have been declining across the globe including Thailand. New emerging diseases are now increasingly discovered. This is the first case report of chytridiomycosis in captive frogs in Thailand. Because Thailand is famous for frog cuisine and frog culture is an important economic activity with high demand for the product in foreign markets. Intensive survey and proper investigation of chytridiomycosis across the country are immediately needed.

Acknowledgements

We thank National Research Council of Thailand (NRCT) for funding and all ZPO veterinarians and herpetologists for supplying frog skin samples

References

Renal Myxozoanosis in a Soft-shell Turtle

N. Pirarat1*, K. Teankum1, N. Chansue2, A. Sailasuta1

1Department of Pathology, 2Department of Veterinary Medicine, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand *Corresponding author E-mail: nopadonpirarat@hotmail.com

Keywords: glomerulo-nephritis, myxozoa, soft-shell turtle

Introduction

Myxozoa are now classified within the kingdom Metazoa. The myxozoa have an elementary type of sexual reproduction and the spores are multicellular in origin (3). Myxozoan infections, usually seen in fish, are occasionally reported in amphibians and chelonians. Renal myxosporidia has been documented in Asian horned frogs in captive zoo (1). Although myxozoanosis has been reported in turtles, renal disease associated with myxozoan infection was rarely reported (2). The objective of this study was to report the clinico-pathological findings of myxozoanosis associated with kidney disease in a soft shell turtle and to characterize the lesions within the affected kidney.

Materials and Methods

A 23-year old male soft shell turtle, 34 kg body weight, had developed clinical signs of severe depression, anorexia, pale mucous membrane, subcutaneous edema, pale soft exudative myopathy, multifocal to diffuse erosions of skin and bedsore wound at the ventral carapace. Abnormal laboratory findings included anemia (<10% hematocrit), decrease blood urea nitrogen (< 9 mg/dl), hypoproteinemia and increase uric acid (1.6 mg/dl). The animal was treated with enrofloxacin (170 mg), Acetar saline solution and Biocatalin. The animal eventually died one day after treatment. The carcass was submitted for necropsy. Tissue samples were fixed in 10% neutral buffered formalin and embedded in paraffin for histopathological investigation. Formalin fixed kidneys were further processed for transmission electron microscopic study.

Results and Discussion

Macroscopically, General carcass appearance showed emaciation. The liver was yellowish color with marked bile retention and bile precipitation. The spleen was slightly enlarged and the kidneys were small. The urinary bladder was empty. Microscopic findings of liver revealed severe panlobular fatty degeneration, moderate infiltration of melanophages containing black pigment, some containing brown pigment. In kidney, multifocal lymphocytic interstitial nephritis and necrosis of the tubular epithelium were obviously seen. The glomeruli were rather thick by pink substance. Some glomerular basement membranes were thickening with irregular appearance. Numerous distal tubules were necrosis and infiltrated by inflammatory cells. In some tubular lumen, clumps of organisms compatible with metazoan spore (2-3μm) were observed. Elongated organisms (2x4 μm), smaller than erythrocytes, were also seen in epithelial cells of renal tubule. Increase amount of heterophils infiltration was remarkable at peri-tubular area. Nephrocalcinosis characterized by an accumulation of dark purple calcified materials in the lumen of renal epithelium were obviously noted. Multifocal lymphoid necrosis and depletions were moderately observed. Electron microscopy revealed various stage of mature spore and developmental stage of myxozoa in the renal tubular epithelium. Based on the clinical and pathological data, the animal died from chronic renal failure caused by myxozoan infection.

Acknowledgement

This study was supported by a grant for Development of New Faculty Staff, Chulalongkorn University (GDNS 51-036-31-004).

References

Microsporidia Infection in Swordtail fish, *Xiphophorus helleri*

A. Ponpornpisit¹, M. Endo², N. Pirarat³*

¹Department of Veterinary Medicine, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand
²Laboratory of Fish Health Management, Tokyo University of Marine Science and Technology, Japan
³Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

*Corresponding author: nopadonpirarat@hotmail.com

**Keywords:** microsporidia, swordtail fish

### Introduction

Thailand produces and exports a lot of ornamental fish every year. The fish were exported to many countries including Hong Kong, USA, China, Singapore and Germany (1). However, exported ornamental fish usually encounter with some infectious problems. Microsporidian infection has been reported in many species including cyprinid (4), neon, tetra, danios, barb and gold fish (3). Amazon River was the place where the original infection fish has been found. Owing to an improper elimination or less intensive examination, the disease rapidly contaminated worldwide (3). Although the infected fish had no sign of stress or distress and the infection and the mortality rate were very low, the whitish patch under its skin made it less beautiful and customer will refuse to buy. This problem gave negative impact to the whole ornamental fish business. Thus highly concerned and good performing practice is immediately needed. This is the first report of an outbreak of Microsporidian infection in swordtail fish in an ornamental fish farm in Thailand.

### Materials and Methods

Visible and invisible signs of infected swordtail fish (*Xiphophorus helleri*), sludges and insects from the same infected pond in Ratchaburi province, Thailand were sampled to the Faculty of Veterinary Science, Chulalongkorn University, Thailand and the Faculty of Marine Science, Tokyo University of Marine Science and Technology, Japan for intensive study. The visible infection rate was around 10% and the mortality rate was about 1%. There is no visible sign in early stage of infection but single or multiple whitish areas under fish skin and/or abnormal swimming posture can be detected in chronic stage. The fish were subjected to study for pathology and histopathology while other samples were observed for a possible chance to be microsporidian reservoir. Study techniques include fresh tissue squash with and without Giemsa staining and observed under light microscope. Samples were fixed in 10% formalin and routinely processed for histopathological study.

### Results and Discussion

Gross macroscopic finding revealed single or multiple whitish patchy areas with well demarcated in the trunk muscles. Microscopically, an infiltration of lymphocytes and eosinophilic granular cells at the intermuscular bundles were occasionally seen. Muscular dystrophy, fibrosis and fragmentation of muscle fiber were detected. Granulomatous formation with fibrotic encapsulation was obviously seen. Macrophages-laden microsporidia were demonstrated in trunk muscle, head and trunk kidney. Multiple stages of microsporidian and variables large spores packed in an amorphous sporophorous vesicle wall were largely demonstrated in the affected muscle, thus characterized as *Pleistophora* spp. (2). Asexual stages of parasites were detectable in tubular epithelium in trunk kidney. There were no microsporidia in brains, livers, swim bladders, gonads, spleen, gills and hearts. Interestingly, microsporidia spores were also detected in sludges, snails and insects at the same pond, suggesting the disease might be widely transmitted by these potential carriers.

### Acknowledgement

This work was supported by a grant from Chulalongkorn University and Japan Society for the Promotion of Science.

### References

Acute Oral Toxicity Test of Colloidal Silver Nanoparticles

T. Kaewamatawong1*, W. Banlunara1, S. Ekgasit2, P. Maneewattanapinyo2

1Department of Pathology, Faculty of Veterinary Science, 2Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand 10330  *Corresponding author: Theerayut71@hotmail.com

Keywords: acute, oral, colloidal silver nanoparticles, mouse toxicity

Introduction

Engineered nanoparticles (NP) are defined as materials produced within the range of 1-100 nm in length or diameter. Nanoparticles have the increased structural integrity as well as unique physical and chemical properties (3). Although the applications and benefits of these engineered nanomaterials are extensively and currently being widely used in modern technology, there is a severe lack of information concerning the human health and environmental implications of occupational exposure during the manufacturing and handling process (2). Silver nanoparticles (Ag-NPs) have been known to have inhibitory and bactericidal effects as well as the effective in retarding the growth of mold, harmful spores and germs (1). Ag-NPs are found to be a popular constituent in health applications and ink industry. Despite the varied uses of these Ag-NPs in many commercial products that launched into the market recently, there is a lack of information on the basic toxicity of silver nanoparticles. Thus, the objective of this study is to investigate the acute oral toxicity of silver nanoparticles using the recommended Organization for Economic Cooperation and Development (OECD) guidelines for testing of chemicals for safety evaluation. Furthermore, lethal Dose 50 (LD50) or Toxic Dose 50 (TD50) is evaluated in this study.

Materials and Methods

Particles: Colloidal silver nanoparticles were obtained as a gift from Sensor Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University, Thailand. The Ag-NPs were suspended in water in various concentrations and had a primary particle diameter of 5-20 nm.

Experimental design: The acute oral toxicity of Ag-NPs was evaluated in mice using the up and down procedure (4). Mice of either sex (nine females and nine males, weight: 28-35 g, age: 10-12 weeks) received colloidal Ag-NPs at the limited dose of 5,000 mg/kg (100,000 ppm) orally using a suitable intubation cannula. The animals were observed for toxic symptoms continuously for the first 3 hr after dosing. Finally, the number of survivors was noted after 24 hr and these animals were then maintained for further 14 days with observations made daily. At 1, 7 and 14 days after gavage, six mice in each group were sacrificed. Whole blood was collected for routine clinical pathology and blood chemical parameters including asparate aminotransferase (AST), serum creatinine, cholesterol and total protein. Various organs such as lung, hilar lymph node, heart, liver and kidney were collected in 10% buffered neutral formalin for routine histopathological evaluations.

Results and Discussion

Clinical and general signs: No death was recorded in the 14 days of observation period in the male and female animals given 5000 mg/kg of the colloidal Ag-NPs orally. The animals did not show any significant changes in the general appearance during the 14 days observation period.

Body weight: There were no significant differences in the percentage of weight gain between control and treatment groups of both sexes.

Blood analysis: Routine hematological analysis and leukocyte differential count showed no significant changes in the male and female treatment groups compared to the control groups. The result of blood chemistry study also showed no significant differences in any of the parameters examined in either the control or the animals treated with Ag-NPs.

Tissue analysis: There were no detectable abnormalities on gross findings in any observation time. Histopathological examination of various organs in the control and treated animals showed no remarkable lesions that could be attributed to the effect of oral exposure of Ag-NPs on mice for 14 days observation period.

Conclusion: The results of acute toxicity study indicated that the LD50 or TD50 of the colloidal Ag-NPs is greater than 5000 mg/kg or 100,000 ppm in line with the 5000 mg/kg limit dose recommend by OECD 425 (4). It is therefore concluded that the acute oral administration of colloidal Ag-NPS at 5000 mg/kg body weight for 14 consecutive days to male and female ICR mice did not induce any toxicological effects. However, further long-term or chronic exposure of Ag-NPs should be performed.

Acknowledgement

This work was supported by a grant from The National Research Council of Thailand, 2008.

References

Acute Dermal Toxicity Test of Colloidal Silver Nanoparticles

T. Kaewamatawong1*, W. Banlunara1, S. Ekgasit2, P. Maneewattanapinyo2

1Department of Pathology, Faculty of Veterinary Science, 2Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand 10330  *Corresponding author: Theerayut71@hotmail.com

Keywords: acute, colloidal silver nanoparticles, dermal, guinea pig, toxicity

Introduction

Engineered nanoparticles (NP) are defined as materials produced within the range of 1-100 nm in length or diameter. Nanoparticles have the increased structural integrity as well as unique physical and chemical properties (3). Although the applications and benefits of these engineered nanomaterials are extensively and currently being widely used in modern technology, there is a severe lack of information concerning the human health and environmental implications of occupational exposure during the manufacturing and handling process (2). Silver nanoparticles (Ag-NPs) have been known to have inhibitory and bactericidal effects as well as the effective in retarding the growth of mold, harmful spores and germs (1). Ag-NPs are found to be a popular constituent in health applications and ink industry. Despite the varied uses of these Ag-NPs in many commercial products that launched into the market recently, there is a lack of information on the basic toxicity of silver nanoparticles. Thus, the objective of this study is to investigate the acute dermal toxicity of silver nanoparticles using the recommended Organization for Economic Cooperation and Development (OECD) guidelines for testing of chemicals for safety evaluation. Furthermore, lethal Dose 50 (LD50) or Toxic Dose 50 (TD50) is evaluated in this study.

Materials and Method

Particles: Colloidal silver nanoparticles were obtained as a gift from Sensor Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University, Thailand and had a primary particle diameter of 5-20 nm. The Ag-NPs were suspended in water in various concentrations.

Experimental design: Male guinea pigs (500-650 g) were randomly divided into 3 groups containing 3 animals each in the following manner: group 1, distilled water (vehicle control); group 2 and group 3, 50 and 100,000 ppm of colloidal Ag-NPs, respectively. All treated groups received the above chemicals at 2 ml. The procedure used for determining the dermal toxicity of the above chemicals followed the procedures as recommended and documented by OECD 434; acute dermal toxicity-fixed dose procedure (4). Briefly, the Ag-NPs were dissolved in distilled water and applied to a shaved area of skin, approximately 7x10 cm2. The chemical was left in contact with the skin with a porous gauze dressing and non-irritating tape for 24 hours. All animals were observed for toxic symptoms continuously at 1, 3, 7 and 14 hr after dosing. After 24-hr exposure period, any residue was removed by washing with distilled water. The number of survivors was noted after 24 hr and these animals were then maintained and observed for toxic signs for further 14 days with observations made daily. At 1, 3, 7 and 7 days after exposure, skin biopsy was performed for routine histopathological evaluations. All animals were sacrificed after a 14 day observation period and collected the skin for histopathological examination.

Results and Discussion

Clinical and gross findings: All control and treated animals, there were no exposure-related clinical signs in any observation time. Grossly, the control, 50 and 100,000 ppm colloidal Ag-NPs did not show any significant changes in the general appearance and skin condition during the 14 days observation period (Fig. 1).

Histopathology: No significant lesions were observed in the skins from treatment groups compared to the control animals at all observation times (Fig. 2).

The results of acute dermal toxicity study indicated that the LD₅₀ or TD₅₀ of the colloidal Ag-NPs is greater than 100,000 ppm. It is therefore concluded that the acute oral administration of colloidal Ag-NPs at 50 or 100,000 ppm for 14 consecutive days did not induce any toxicological effects. However, further long-term or chronic repeated exposure of Ag-NPs should be performed.

Acknowledgement

This work was supported by a grant from The National Research Council of Thailand, 2008.

References

Acute Eye Irritation and Corrosion Test of Colloidal Silver Nanoparticles  

T. Kaewamatawong1*, W. Banlunara1, S. Ekgasit2, P. Maneewattanapinyo2  

1Department of Pathology, Faculty of Veterinary Science, 2Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand 10330  *Corresponding author: Theerayu71@hotmail.com  

Keywords: acute, colloidal silver nanoparticles, eye, irritation, mouse  

Introduction  
Engineered nanoparticles (NP) are defined as materials produced within the range of 1-100 nm in length or diameter. Nanoparticles have the increased structural integrity as well as unique physical and chemical properties (3). Although the applications and benefits of these engineered nanomaterials are extensively and currently being widely used in modern technology, there is a severe lack of information concerning the human health and environmental implications of occupational exposure during the manufacturing and handling process (2). Silver nanoparticles (Ag-NPs) have been known to have inhibitory and bactericidal effects as well as the effective in retarding the growth of mold, harmful spores and germs (1). Ag-NPs are found to be a popular constituent in health applications and ink industry. Despite the varied uses of these Ag-NPs in many commercial products that launched into the market recently, there is a lack of information on the basic toxicity of silver nanoparticles. Thus, the objective of this study is to investigate the acute eye irritation and corrosion of colloidal silver nanoparticles using the recommended Organization for Economic Cooperation and Development (OECD) guidelines for testing of chemicals for safety evaluation.

Materials and Methods  
Particles: Colloidal silver nanoparticles were obtained as a gift from Sensor Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University, Thailand and had a primary particle diameter of 5-20 nm. The Ag-NPs were suspended in water in various concentrations.  
Experimental design: Male guinea pigs (500-650 g) were randomly divided into 2 groups containing 4 animals each in the following manner: group 1, 50 ppm of colloidal Ag-NPs and group 2, 5,000 ppm of colloidal Ag-NPs. The procedure used for determining the ocular toxicity of the above chemicals followed the procedures as recommended and documented by OECD 405; acute eye irritation and corrosion (4). Briefly, the 0.1 ml of colloidal Ag-NPs suspension was placed in the conjunctival sac of one eye of each animal after gently pulling the lower lid away from the eyeball. Another eye, which remains untreated, serves as a control by instilling with 0.1 ml of distilled water. All animals were observed for toxic symptoms continuously at 1, 12, 24, 48 and 72 hr after dosing. The eye reactions of iris, conjunctivae, cornea and chemosis were graded following the grading system of OECD 405 guideline. The animals were then maintained and observed for toxic signs for further 14 days with observations made daily.

Results and Discussion  
Clinical and general signs: The animals from control and treated animals did not show any toxic signs in the clinical and general appearance during the 14 days observation period.  
Ocular reactions: No any significant lesion was observed in the control and 50 ppm Ag-NPs treated animals throughout the observation period (Fig. 1). During first 24 hr observation time, some animals from 5,000 ppm Ag-NPs treated group showed grade 1 of conjunctivae irritation, which some blood vessels hyperemia in conjunctivae were observed (Fig. 2). However, no any sign of eye irritation was found in all treated animals after 48 hr post-exposure. The results of acute eye administration of colloidal Ag-NPs at 50 or 5,000 ppm for 14 consecutive days did not induce any toxicological effects. However, the animals from 5,000 ppm groups showed transient mild conjunctival irritation at early 24 hr post-exposure. It is therefore concluded that the acute ocular toxic dose of the colloidal Ag-NPs might be greater than 5,000 ppm. Further long-term or chronic repeated exposure of Ag-NPs should be performed.

Acknowledgements  
This work was supported by a grant from The National Research Council of Thailand, 2008.

References  
Acute Pulmonary Toxicity Caused by Single Intratracheal Instillation of Colloidal Silver Nanoparticles in Mice

T. Kaewamatawong1*, W. Banlunara1, S. Ekgasit2, P. Maneewattanapinyo2

1Department of Pathology, Faculty of Veterinary Science, 2Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand 10330 *Corresponding author: Theerayut71@hotmail.com

Keywords: acute, colloidal silver nanoparticles, lung, mouse, toxicity

Introduction

Engineered nanoparticles (NP) are defined as materials produced within the range of 1-100 nm in length or diameter. Nanoparticles have the increased structural integrity as well as unique physical and chemical properties (3). Although the applications and benefits of these engineered nanomaterials are extensively and currently being widely used in modern technology, there is a severe lack of information concerning the human health and environmental implications of occupational exposure during the manufacturing and handling process (2). Silver nanoparticles (Ag-NPs) have been known to have inhibitory and bactericidal effects as well as the effective in retarding the growth of mold, harmful spores and germs (1). Ag-NPs are found to be a popular constituent in health applications and ink industry. Despite the varied uses of these Ag-NPs in many commercial products that launched into the market recently, there is a lack of information on the basic toxicity of silver nanoparticles. Moreover, data of the pulmonary pathological effects of Ag-NPs have not been reported to our knowledge. The purpose of this study is to describe acute pulmonary pathological effects caused by intratracheal exposure to various doses of Ag-NPs.

Material and Methods

Particles: Colloidal silver nanoparticles were obtained as a gift from Sensor Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University, Thailand and had a primary particle diameter of 5-20 nm. The Ag-NPs were suspended in water in various concentrations.

Experimental design: 60 Male ICR mice were single intratracheally instilled with 50 μl aqueous suspensions of 20, 200, 2000 or 20,000 ppm of Ag-NPs suspended in distilled water. The control groups of mice were instilled with 50 μl of distilled water. At 1, 3, 7 and 14 days after instillation, the animals in each group were sacrificed. Various organs such as lung, hilar lymph node, heart, liver and kidney were collected in 10% buffered neutral formalin for routine histopathological evaluations.

Results and Discussion

Clinical and gross findings: In control, 20 and 200 ppm of Ag-NPs treated animals, there were no exposure-related clinical signs in any observation time. Some mice in 2,000 and 20,000 ppm treated animals showed a sign of dyspnea shortly after instillation. However, this sign was recovered after 6 hr post-exposure. Grossly, instillation of 20 and 200 ppm Ag-NPs treated animals caused mild congestion and edema in lung compared to the control groups. In both 2,000 and 20,000 ppm Ag-NPs treated animals, tiny pin-head sized or patchy black brown foci were scattered in lung lobes throughout the experiment.

Histopathology: At 1 day after instillation, accumulation of free aggregated particles was found in the alveoli and bronchiolar lumens of all treated groups. Some of aggregated particles were present within alveolar macrophages, and occasionally present within alveolar epithelial cells with increasing number of cells in alveolar wall (Fig. 2A). The animal instilled with 2,000 and 20,000 ppm Ag-NPs had a moderate to severe accumulation of Ag-NPs laden alveolar macrophages and inflammatory cells in lung parenchyma. At 3 days after instillation, moderate to severe focal alveolitis characterized by accumulation of numerous active AMs, particle-laden AMs, inflammatory cells was observed (Fig. 2B). Changes in the lungs of mice killed at 7 and 14 days post-exposure were distributed to the appearance of the alveolitis with some necrotic areas (Fig 2C). The magnitude lesions in 20,000 ppm groups were greater than 2,000 ppm groups.

An acute pulmonary instillation Ag-NPs above 2,000 ppm for 14 consecutive days can induce lung inflammation and tissue injury in a dose dependent manner.

Acknowledgement

This work was supported by a grant from The National Research Council of Thailand, 2008.

References

Pathological Investigations on Tumors of Ornamental Fish in Thailand

P. Komane1, P. Ruangwilaisup1, J. Chaiworawitsakul1, N. Chunsue2, S. Larcharoj3, A. Sailasuta3*

16th year student academic year 2009, 2Department of Veterinary Medicine, 3Department of Veterinary Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, 10330 Thailand

*Corresponding author: achariya.sa@chula.ac.th

Keywords: ornamental fish, pathology, Thailand, tumors

Introduction

Nowaday, the incidence of tumors in domestic animals has been increased due to many factors. In veterinary practice there were many studies reported on the classification and diagnosis of the tumors. While, there were a few informations on tumors in aquatic animals. Fish develop tumors and cancer, much like humans and other animals. Tumors in fish can also be due to some predisposing factors such as carcinogenic compounds, virus, irritants, oncogenes and parasites (1). Most tumors are seen as bumps or lump under the fish skin. The location and signs of tumor can be different for each case and type of tumors (2, 3). The aim of this report is to investigate the pathological classification on tumors of ornamental fish.

Materials and Methods

A retrospective study of forty tumor biopsied samples from ornamental fish were collected from department of Veterinary Pathology, Faculty of Veterinary Science, Chulalongkorn University during 2000-2009. The specimens were fixed in 10% formalin, routinely embedded in paraffin and sectioned. For light microscope, the sectioned were stained with HE and serial sections were subsequently observed by selected special stains i.e Alcian blue, Masson trichrome and Von kossa (4). The Immunohistochemistry, avidin-biotin complex; using vimentin and cytokeratin antibody was also applied.

Results and Discussion

The ornamental fish in this study are goldfish, 72.5% (29/40); carp, 10% (4/40); flowerhorn, 7.5% (8/40); and red tailed catfish, arowana and shark, 2.5% (1/40) of each respectively. Regards to the tumor site, the lesions was commonly found on skin and head area as well as the age of goldfish is about 2-4 years. Upon the histopathology, the tumors were classified into 3 groups as soft tissue tumor; 62.5% (25/40) epithelial tumor; 35.0% (14/40) and hemopoietic tumor; 2.5% (1/40) respectively. The details of these groups has shown in Table 1 and Fig. 1. These results are in agreement with fibromas are the common tumors in goldfish (2). It is possible to apply keratin antibody in case of skin tumor. While, the mesenchymal origin tumor as fibromas were negative immunoreactivity for vimentin (3). Many study of tumor in fish tumors inductive by virus such as fibromas (1-3). It is suggested to do further study on the relationship of fibroma and the viral infection which should be benefit for prevention and control of tumor in aquatic animals.

Table 1: The classification of tumors (n=40)

<table>
<thead>
<tr>
<th>Tumors type</th>
<th>Cases</th>
<th>Tumors type</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibroma</td>
<td>16</td>
<td>Papilloma</td>
<td>10</td>
</tr>
<tr>
<td>Fibrosarcoma</td>
<td>2</td>
<td>Melanoma</td>
<td>3</td>
</tr>
<tr>
<td>Osteoma</td>
<td>2</td>
<td>Ovarian adenoma</td>
<td>1</td>
</tr>
<tr>
<td>Chondroma</td>
<td>2</td>
<td>Lymphoma</td>
<td>1</td>
</tr>
<tr>
<td>Hemangioma</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neurofibroma</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1 (A) The neoplastic cells of fibromas are spindle-shaped cells, formed many wave and had mildly collagen fibers (Masson trichrome) (B); (C) The skin tumor was in papillomatous pattern of epidermis and the cytokeratin antibody was positive reactivity in epidermal structure of fish (IHC, DAB) (D); (E) The soft tissue tumor of chondroma was in cartilaginous structure with bone matrix, positively stained in blue color (alcian blue) (F) bar 50 μm

Acknowledgements

Senior project fund, academic year 2009, Faculty of Veterinary Science, Chulalongkorn University.

References

Generalized Disseminated Intravascular Coagulation Caused by Alpha-hemolytic Streptococcus spp. in Capybara (Hydrochaeris hydrochaeris)

A. Sommanustweechai*, W. Banlunara2, K. Kanjanapitakkul3, S. Sanannu3, B. Siriaroonrat1

1Conservation Research and Education Centre, Zoological Park Organization, Bangkok, Thailand 10300
2Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand 10330
3Dusit Zoo, Zoological Park Organization, Bangkok, Thailand 10300
*Corresponding author: angangkana@yahoo.com

Keywords: alpha-hemolytic Streptococcus spp., capybara, DIC

Introduction
Capybara (Hydrochaeris hydrochaeris) is the largest living rodent. It is a semi aquatic mammal of Central and South America, a member of the family Hydrochoeridae. Capybaras are social animals, usually found in groups controlled by a dominant male. Capybaras become sexually mature at 15-18 months. Vascular thrombosis may have several common causes including bacterial infection releasing tissue procoagulants. Streptococci can often be considered as commensal flora, however it can also cause life-threatening infections.

Materials and Methods
A captive 1 year-old-female capybara was previously mated for many times presented with both hindlimbs lameness, treated initially with nonsteroidal anti-inflammatory drugs and antibiotics. Radiographic examination was not found evidence of fracture and displacement. She was shocked on the 4th days after the signs. Both hind limbs ischemia was seen and developed gangrene necrosis, and died 2 hours after admission.

Results and Discussion
Both hind limbs were cyanotic, necrotic with deep gangrene and sloughing of tissues (Fig. 1A). Macroscopic findings showed obvious signs of generalized thrombosis in the heart, vena cava, lungs and femoral vessels (Fig. 1B), hemorrhages of lung and adrenal and hemorrhagic ulcerative gastritis. Histopathology revealed generalized thrombosis and DIC in various organs; mainly the heart, lungs, kidneys and both hindlimbs (Figs. 2, 3). The stomach also showed severe diffuse multifocal ulcerative, hemorrhagic fibrino-purulent gastritis. Alpha-hemolytic streptococci spp. was isolated from blood, lung and thigh muscle by bacterial cultures. Laboratory test results suggested sepsis with disseminated intravascular coagulation (DIC) and alpha-hemolytic streptococci were isolated from blood culture.

DIC associated with alpha-hemolytic streptococcus infection was finally diagnosed as the definite cause of death. In human being, alpha-hemolytic streptococcus commonly causes serious complications as iatrogenic meningitis after spinal-epidural anesthesia (1). Generalized vascular thrombosis is a well recognized complication of various causes, including septicemia. DIC is difficult to diagnose, rapid and sensitive laboratory diagnosis could help in treatment success. The underlying disease determines the management of DIC; conventional anticoagulant therapy is most commonly used in initial stages of DIC (2). In conclusion, Streptococcus infection can induce DIC in capybara. Success in management of DIC should depend on the early diagnosis.

References
Histopathological and Immunophenotyping Classification of Spontaneous Canine Lymphoma

J. Chayapong¹, J. Jongchalermchai¹, T. Thongruk¹, N. Manachai², S. Wangnaitham², S. Techangamsuwan², A. Rungsipipat²*

¹6th year student academic year 2009, ²Department of Veterinary Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, 10330 Thailand  *Corresponding author: anudep.r@chula.ac.th

Keywords: B cell, canine, immunophenotype, lymphoma, T cell

Introduction
Lymphoma is a neoplasm that is commonly diagnosed in dogs, accounting for up to 24% of all canine neoplasms (1). Lymphoma in dogs has received considerable veterinary interest because it is the one of the most treatable cancers in small animal medicine (2). Many attempts have been made to identify tumors and patient factors that may act as useful predictors of response and prognosis for canine lymphoma. Previously studies agreed that immunophenotype was an important prognostic indicator with B cell lymphomas having a better prognosis than T cell lymphomas. The purpose of this study was to study determine the histological characteristics and the immunophenotype of the canine lymphoma in Bangkok metropolitan using the Kiell's classification.

Materials and Methods
This study consisted of 40 formalin-fixed paraffin wax-embedded tissue sections from dogs affected with lymphoma, diagnosed during 2002-2008 at the Department of Veterinary Pathology, Faculty of Veterinary Science, Chulalongkorn University. All tissue samples were examined by histological and immunophenotypic characteristics. HE stained sections were reviewed and classified according to the anatomical classification of canine lymphoma. Immunophenotype was determined by immunohistochemical reaction to CD3 and IgM antibodies for T and B cell lineages.

Fig. 1 Small cell lymphocytic lymphoma (HE, x400)  Fig. 2 Small cell prolymphocytic lymphoma (HE, x400)  Fig. 3 Centroblastic monomorphic lymphoma (HE, x400)  Fig. 4 Centroblastic polymorphic lymphoma (HE, x400)  Fig. 5 Plosmorphic large cell lymphoma (HE, x400)  Fig. 6 Immunoblastic lymphoma (HE, x400)  Fig. 7 T cell lymphoma immunolebelled with CD3 antibody (IHC, x400)  Fig. 8 B cell lymphoma immunolebelled with IgM antibody (IHC, x400)
Results and Discussion

The anatomical classification of canine lymphoma revealed mostly multicentric form (Table 1.). Histopathology categorized canine lymphoma 40 cases into 2 groups as shown in Table 2. Both Immunophenotyping cases were 50% of T and B cell lymphoma. Low and high grade lymphoma accounted 17 and 23 cases, respectively. Upon the Kiel’s classification, the lymphomas could be classified into subtypes as shown in table 2.

The relationship between disseminated or multicentric lymphoma and majority of T cell subtype tend to show a malignancy behavior.

References

In Vitro Antiproliferation Activity of Temulawak (Curcuma xanthorrhiza Roxb.)
Ethanol Extract on YAC-1 and HeLa Tumor Derived Cell Lines

B.P. Priosoeryanto1,2*, E.J. Stephani1, R. Sari1, L.K. Darusman2, E.D. Purwakusumah2, W. Nurcholis2, R. Tiuria1,3

1Division of Veterinary Pathology, 2Division of Veterinary Parasitology, Faculty of Veterinary Medicine;
3Biopharmaca Research Center, Bogor Agricultural University (IPB)-Indonesia
*Corresponding author: bpontjo@indo.net.id

Keywords: antiproliferation, Curcuma xanthorrhiza, ethanol extract, in vitro, HeLa, YAC-1

Introduction
A tumors or neoplasm can be defined as a disturbance of growth characterized by excessive, abnormal and uncontrolled proliferation of transformed or altered tissue at one or more primary points within the host, and frequently at one or more metastatic sites. Natural metabolites especially from plants are widely used for medical purposes. In some Asian countries, the use of plants for traditional medicine in the treatment of some disorders in human and animal is a common practice. C. xanthorrhiza known as “Temulawak” in Indonesian language is one of 7,000 Indonesiaís medicinal plants out of 30,000 plants found in Indonesia; this number accounted as a 90% of Asiaís medicinal plants (1) Ethanol extracts of the root of C. xanthorrhiza (Fig. 1) was known had an antiproliferation activity on canine tumor-derived cell lines (2). This plant extract gave a significant inhibition of cell growth activity on both canine tumor cell lines. The aim of this study is to elaborate the anti-proliferation effect of 70%-ethanol extracts from C. xanthorrhiza on tumor-derived cell lines in order to find the anti-tumor drugs for medical purposes both in human and animal.

Material and Methods
Brine Shrimp Lethality Test: Ten larvae of Artemia salina on 18 vials each were used (5 concentrations of extracts and one control with 3 replicates). After 24 hours of extracts treatment, the dead A. salina was counted. The data were processed statistically using Probit Test.

Cell Culture: Cell lines were cultivated in a 24-well culture plate on DMEM-F12 supplemented with 10% FCS and antibiotics (100 IU/mL penicillin, 100 g/mL streptomisin) with density of 10^5 cell/mL. Cells were then exposed to 6 different concentration of the extract i.e. 0, 15, 30, 45, 60, 75 ppm; and doxorubicin was used as a control positive. Treated cells were plated in 3 replicates. Cells were then incubated at 37°C with 5% CO₂ in air.

Cell Harvesting & Counting: Cells were harvested after 4 days in culture when confluence was achieved. Total cells from each treatment were counting using a hemacytometer with Trypan Blue dye exclusion and the cell numbers were averaged. The antiproliferation activity was then calculated.

Results and Discussion
Anti-proliferation Activity: Exposed of YAC-1 and HeLa cells with gradual concentrations of C. xanthorrhiza extract resulted in the increasing of the cell growth inhibition (Fig. 2), this condition indicated that there was an antiproliferation activity of this extract to these both cell lines.

The degree of this activity was varied in each cell lines. The highest anti-proliferation activity of C. xanthorrhiza ethanol extract on each cell lines were 70.0% on YAC-1 cell line and 37.41% on HeLa cell line (Fig. 3). This activity was occurred on the dose of 75 ppm.
C. xanthorriza had a main bioactive substaces of curcuminoid and others (3). Curcuminoid give the root a yellow colour and had an anti-bactery, anti-cancer, anti-inflamation, anti-oxidant and hypocholestemic (4). The other components in C. xanthorriza were consists of camphor, mirsen, xanthorizol, β-curcumin, arcurcurmin, isofuranogermakren and p-toluilmethylcarbinol (5). Xanthorrizol combined with curcumin were the main substances that acts as potential bioactive compound of C. xanthorriza (3). Ethanol extract of C. xanthorriza has an activity in inhibiting the growth of MCA-B1 and MCM-B2 derived canine-tumor cell lines with inhibition ranging from 70-75% at the extract concentration of 75 ppm (2). Curcumin was reported could inhibit the cell proliferation of several tumor cells i.e. HL-60, human leukemia, depend on the dose and time exposure (6). Curcumin inhibited cell proliferation by stimulating the apoptotic mechanism through mitochondrial pathway involving activation of Caspase-8, BID cleavage, Cytochrome C release dan Caspase-3. Curcumin also inhibited the induction of nitrate oxide synthesis within the activated-macrophages. Curcumin showed their activity on anti-cancer by reducing the number of nitrate oxide or iNOS (inducible nitric oxide synthase) which known as one of the initiator of the tumor formation. NF-kappa-B is involve in the induction of iNOS, caused oxidative stress, which know as one of the tumor initiator. Curcumin acts by inhibit the phosphorylation and degradation of kappa-B-alpha inhibitor through a mechanism by inhibiting the activation of NF-kappaB, where the result will decreasing the transcription of iNOS gene (7). Curcumin could inhibit lipoxygenase (LOX), cyclooxygenase (COX)-1 dan COX-2 as well as lipopolysacharides that will terigered the COX-2 expression (6). In tumor cells, the excessive expression of COX-2 which resulted in the over production of prostanoid will caused increasing the proliferation and inhibit the process of apoptosis (8). Increasing in cell proliferation is occured due to activation of several oncogene that involved in the mitogenic signal such as Ras oncogene. Inhibition on the apoptotic process is due to the effect of the excessive expression of Bcl-2 oncogene. Inhibition of COX caused the prevention of excessive prostanoid production by curcumin and resulted in the decreasing of inflammation effect, preventing tumor cell proliferation and enhance the apoptosis process.

In this pathway, apoptotic process is stimulated by the accumulation of acid arachidonat. Accumulation of this acid will activate sphingomyelinase enzyme which catalyze the production of ceramid from sphingomyelin and finally ceramid will stimulate the apoptotic process. Curcumin also capable in the inhibit the initiation process of the tumor formation due to benzo(a)pirene (8). This chemoprevention effect is due to curcumin has an ability in the inhibit the activity of cytochrome P450 and glutathion-S-transferase which causing the inhibition of activation of benzo(a)pirene as a mutagenic substances.

Xanthorrizol, a sesquiterpen component in C. xanthorriza could increasing apoptotic process on HeLa cells by assayed using a TUNEL method as well as nuclear morphology using a Hoechst 33258 stain (9). Xanthorrizol did not influenced the expression of anti-apoptosis protein (Bcl-2) and viral oncoprotein E6. Xanthorrizol is a substance that functioned as an anti-proliferative and anti-cancer through a mechanism by apoptotic induction of p53 and Bax on the HeLa cells. Based on all findings mentioned above, we concluded that C. xanthorriza root-ethanol extract has an antiproliferation activity on YAC-1 and HeLa tumor-derived cell lines, and this phenomenon could be further studied for the widely used of this plant extract in the combating of tumor disorders both in human and animals.

Acknowledgements

The research was supported by the Ministry of Agriculture, The Republic of Indonesia through KKP3T competitive research grant.

References

1. Indonesia Drugs and Food Agency, 2002.
The Supplementation of *Andrograpis panicula* in the Feed of Lactating Sows Reduce Pre-weaning Mortality and Increase Number of Piglets at Weaning

P. Tummaruk∗, V. Limtrajitt2, A. Kunavongkrit1

1Department of Obstetrics Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, 10330 Thailand. 2Lily FoodAnSci Limited, Bangkok, Thailand  *Corresponding author: Padet.T@chula.ac.th

Keywords: feed intake, herbal medicine, pig, weaning weight

Introduction

The use of herbal medicine as a feed additive in livestock industry is increasing nowadays and many recent researches have been taken into consideration (1, 2). Many types of herbal medicine or plant-extract products have been studied but only a few trials have been completed. *Andrograpis panicula* (*A. paniculata*) is a herbal plant in family Acanthaceae originated from India and Sri Lanka and is widely cultivate in southern Asia. *A. paniculata* is one of the most well known herbs, commonly used to treat infections and some diseases in human for decades. *A. paniculata* is used in traditional medicine in India and some other countries for multiple clinical applications. Andrographolide is a chief constituent extracted from the leaves of the plant and is a bitter water-soluble compound. It has been reported that the plant extract exhibits anti-tumourphoid, antifungal, antihepatotoxic, antibiotic, antimalarial, antihepatitic, antithrombogenic, anti-inflammatory, anti-snake venom and antipyrethric properties (3). It is also generally used as an immunostimulant agent and recently, an anti-HIV activity of the plant has been confirmed. The LD50 in male mice was 11.5 gm/kg, ip. Andrographolide is also attributed with such other activities like liver protection under various experimental conditions of treatment with galactosamine and paracetamol. The hepato-protective action of andrographolide is related to activity of certain metabolic enzymes. Immunostimulatory activity of andrographolide is evidenced by increased proliferation of lymphocyte and production of interleukin 2. Andrographolide also enhanced the tumor necrosis factorα production and CD marker expression, resulting in increased cytotoxic activity of lymphocytes against cancer cells, which may contribute for its indirect anticancer activity. To our knowledge, comprehensive study on the clinical application of *A. paniculata* in pig is limited. The objective of the present study was to evaluate the reproductive performance after the application of *A. paniculata* in post-partum and lactating sows. Reproductive index including pre-weaning mortality rate, number of piglets at weaning and the average daily gain of the piglets were determined.

Material and Methods

Animal and data: The present study was conducted in a commercial swine herd in the eastern part of Thailand during February to August 2009. The study included farrowing records of 1,272 litters. Data including the sows' identities, parity number, farrowing house (A, C, G), farrowing date, weaning date, litter birth weight, number of piglets born alive per litter (BA), litter weaning weight, number of piglets at weaning and the body weight of individual piglets at weaning. Means individual birth was calculated by dividing litter birth weight with BA. Lactation length was defined as the interval between farrowing and weaning. Average daily gain (ADG) was calculated using the following formula: ADG (g/day) = [(Average weaning weight-average birth weight)/lactation length] x 1,000. Pre-weaning mortality rate (PWM) was calculated using the following formula: PWM (%) = [(BA-wean)/BA] x 100. The sows were divided into two groups, i.e., control (n=655 litters) and treatment (n=617 litters). The control sows were fed with conventional lactation feed from one week before farrowing to weaning. The treatment sows were fed with the same lactation feed supplemented with 1,000 ppm of a *A. paniculata* and it’s compound. The herb compound included *A. paniculata*, *Curcuma longa* and *Monordica charantia* (Herbatobmix®, Lily FoodAnSci Limited, Bangkok, Thailand).

Herd management: The gilts and sows in the herd were housed in a conventional open-housing system with a water sprinkler and fan; the boars were kept in an environment with an evaporative cooling system. The gilts and sows were kept in individual stalls during gestation and in individual farrowing pens during lactation. The breeds of the sows were predominantly crossbred Landrace×Yorkshire, and were mainly bred with hybrid boars (PIC® Siam Ltd., Thailand). The gilts and sows received water up to ad libitum via water nipples. The feed was provided twice a day (about 1.5-3.5 kg/d during gestation and 5.0-7.0 kg/d during lactation). The feed was a rice-corn-soybean-fish base containing 15-18% crude protein, 2,900-3,200 kcal/kg metabolisable energy and 0.8-1.0% lysine.

Statistical analyses: The statistical analyses were carried out using SAS (SAS 2002). Descriptive statistics were conducted for all parameters. Frequency analysis and chi-squared test were used to analyze proportional data, i.e., proportion of lactation failure sows. Continuous data, i.e., BA, number of piglets at weaning, piglets weaning weight, ADG, litter weight gain and PWM, were analyzed using the general linear model procedure of SAS (PROC GLM). The statistical models included group (control versus treatment), farrowing house (A, C, G) and interactions between group and farrowing house. Lactation length (days) were included in the statistical model as a
covariance variable. Least-square means were obtained from each class of the factors and were compared using a least-significant-difference test. A probability value of $p<0.05$ was regarded to be statistically significant.

**Results and Discussion**

On average, BA, number of piglets at weaning, lactation length, ADG, litter weight gain and pre-weaning mortality were 10.2±2.4 piglets/litter, 9.2±2.7 piglets/litter, 24.6±1.3 days, 200.0±40.8 g/day, 46.2±13.9 kg and 8.6%, respectively. The numbers of litters weaned from the farrowing house A, C and G were 531, 334 and 407 litters, respectively. The frequency distribution of an individual piglets weaning weight is demonstrated in Figure 1. The number of sows that had lactation failure was 34/655 sows (5.2%) and 4/617 sows (0.7%) in the control and treatment group, respectively ($p<0.001$). The proportion of lactation failure were 15/531 (2.8%), 17/334 (5.1%) and 6/407 (1.5%) in farrowing house A, C and G, respectively ($p=0.01$). On average, the numbers of piglets born alive per litter were 10.2, 10.0 and 10.4 piglets/litter ($p=0.06$) and the numbers of piglets at weaning were 9.3, 8.6 and 9.4 piglets/litter ($p<0.001$) in farrowing house A, C and G, respectively. Across the farrowing house, the supplementation of the herbal compound significantly improved number of piglets at weaning, litter weight gain and pre-weaning mortality rate. All parameters measured in the control and the treatment groups are demonstrated in Table 1. Numbers of piglets at weaning and litter weight gains in control and treatment groups by farrowing house are demonstrated in Fig 2 and 3.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Treatment</th>
<th>Different</th>
</tr>
</thead>
<tbody>
<tr>
<td>BA</td>
<td>10.1±2.3</td>
<td>10.3±2.5</td>
<td>+0.2</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>1.6±0.2</td>
<td>1.7±0.3</td>
<td>+0.1</td>
</tr>
<tr>
<td>Lactation (day)</td>
<td>24.7±1.3</td>
<td>24.4±1.2</td>
<td>+0.3</td>
</tr>
<tr>
<td>Wean$^2$</td>
<td>8.5±2.7</td>
<td>10.0±2.4</td>
<td>+1.5$^{**}$</td>
</tr>
<tr>
<td>Weaning weight (kg)</td>
<td>6.6±1.1</td>
<td>6.4±1.0</td>
<td>-0.2$^*$</td>
</tr>
<tr>
<td>Litter weight gain (kg)</td>
<td>44.7±13.4</td>
<td>48.1±14.4</td>
<td>+3.3$^{***}$</td>
</tr>
<tr>
<td>PWM$^3$ (%)</td>
<td>14.6</td>
<td>2.2</td>
<td>-10.4$^{***}$</td>
</tr>
</tbody>
</table>

$^1$BA, number of piglets born alive/litter; $^2$Wean, number of piglets at weaning; $^3$PWM, pre-weaning mortality, $^{**}, p<0.01, ^{***}, p<0.001$

**References**

Diagnostic Cytology in Veterinary Clinical Medicine

H. Sakai

Laboratory of Veterinary Pathology, Department of Veterinary Medicine, Faculty of Applied Biological Sciences, Gifu University, Japan, shiroki@gifu-u.ac.jp

Cytologic examination is a valuable diagnostic aid, and the sampling and preparation procedures used for it are easy, rapid, inexpensive, and minimally invasive. Therefore, it continues to expand as one of the essential tools in veterinary clinical medicine. While cytologic preparation is easy, the quality of the specimen is one of the major factors affecting the diagnostic value of the sample. Moreover, from the viewpoint of diagnosis, there are many differences (appearance, size of cells, etc.) between cytological and histopathological results although the technique used for morphological evaluation is similar in both. It is important for cytopathologists, histopathologists, and even clinicians to understand these differences in order to make an accurate and useful diagnosis.

Sample sources and preparation

The specimens commonly used for cytologic examination are various masses and fluids, prostate gland, lymph nodes, liver, and bone marrow. Rectal and vaginal scrapes are also used. Fine needle biopsy (FNB) is one of the methods used for sampling mass lesions. Intra-abdominal and intra-thoracic lesions can also be targeted accurately if ultrasound- or CT-guided FNB is used. Stamp smear of the core or punch biopsy specimens can be performed to find out whether the sampling was successful.

Basic cell types, infectious agents, and artifacts

Epithelial cells: They have a round nucleus and round to polyhedral cytoplasm. The cytoplasmic borders are relatively distinct. In cytologic smears, the cells are observed to attach to each other and form clusters. In particular, mature cornified squamous cells tend to become discrete.

Mesenchymal cells: These cells have spindle-shaped to polyhedral cytoplasm and spindle-shaped to oval nucleus. The cytoplasmic borders are indistinct. The extracellular matrix (ECM), which is stained pink to magenta, is often observed among these cells.

Discrete round cells: These cells originate from hematopoietic cells and include white blood cells such as mast cells and histiocytes. These cells are round in shape and are not attached to each other. ECM is seldom observed.

Infectious agents: Bacteria are observed more distinctly in cytologic specimens than in histopathological specimens. The shape, size, and location (intra- or extra-cytoplasmic) of the bacteria can be observed clearly in the cytologic specimens. Mycobacterium has a unique appearance and appears as non-stained filaments in histiocytes, although special staining methods are required for histopathological examination. Fungi and algae (e.g., Prototheca spp.) can also be identified easily because of their cell wall, which does not get stained. Fungal classification is mainly based on the growth forms seen in tissues (hyphae or yeast-like shape) and the method of proliferation (budding or endosporogenesis).

The morphology of protozoa varies depending on the stage of growth. Viral particles cannot be observed in cytologic specimens; however, we can often observe them in the form of viral inclusion bodies in various cells.

Artifacts: Artifactual changes and materials sometimes confuse cytopathologists. Common artifactual changes include the rupture of cells and chromatin strands. Immature cells, including lymphoblasts and germ cells, are easily destroyed. The appearance of naked nuclei, wherein the nucleus is not surrounded by cytoplasm, is the most common artifactual change observed, and cytopathologists must be careful not to misidentify them as malignant cells because the nucleoli often show up prominently. Glove powders, ultrasound gel, squames, and cotton fibers
are frequent contaminants. Staining precipitates bear a resemblance to bacteria and must be differentiated from them.

**Diagnostic approach for mass lesions**

For the diagnosis of mass lesions, a general approach for the interpretation of cytologic findings involves first determining whether the specimen is neoplastic or inflammatory and, then, the type of neoplasia or inflammation present. Inflammations are subclassified into acute, subacute/chronic suppurative, and granuloma. If many degenerate neutrophils are observed, it is suggestive of an acute bacterial infection, especially one caused by gram-negative organisms. If non-degenerate neutrophils are predominant, it may be a case of chronic suppurative (bacteria are not observed) or immune-mediated inflammation. Eosinophilic inflammation suggests an allergic reaction or a parasitic infection. Smears from granulomas contain many macrophages and/or histiocytes (>50% of the total nucleated cells). Occasionally, multinucleated giant cells (foreign body giant cells) are seen scattered in a smear.

Neoplasms are subdivided into epithelial tumors, spindle cell tumors, and discrete round cell tumors according to the classification of basic cell types. Although squamous cell carcinomas are epithelial tumors, the cells undergoing keratinization in these tumors have pale-to-sky-blue cytoplasm and tend to detach from each other. Some discrete round cell tumors, i.e., mastocytoma can be diagnosed definitively. However, some anaplastic carcinomas or sarcomas might also show cells with a discrete round shape.

**Criteria for diagnosis of malignancy**

**Nuclear criteria:** Anisokaryosis, a variable and usually increased nucleus-to-cytoplasm ratio, abnormally clumped chromatin, and large multiple irregularly shaped nucleoli are important nuclear changes seen in malignant cells. An increased number of mitotic figures are commonly observed in neoplastic cells; however, they also are observed in normal actively proliferating cells. Multiple nuclei might be observed in neoplastic cells. Abnormal mitoses and nuclear molding are nuclear changes observed in malignant cells. Cytoplasmic criteria: The cytoplasmic criteria for diagnosis of malignancy are less important than the nuclear criteria. Increased basophilia and vacuolation are commonly observed in malignant cells.

**Structural and other criteria:** Cellular crowding is a common feature of aspirates obtained from malignant epithelial tissue. Neoplastic epithelial cells sometimes replicate without dividing, resulting in long chains of attached cells. In the case of FNB specimens, a higher cell count is seen in the case of malignant tumors than benign tumors. Necrotic back ground is often observed in the case of malignant tumors.

**Disadvantages of diagnosis on the basis of cytologic examination**

**Low cellularity specimens:** It is difficult to obtain cells from ECM-rich tumors such as fibromas and scirrhous carcinomas, and if there are only a few cells that can be observed on the slides, the possibility of a neoplasm cannot be excluded. Thus, when the cells are not obtained even after performing FNB with aspiration, it is necessary to carry out histopathological examination.

**Granuloma and fibrous inflammation vs. neoplasm:** Granulomas are proliferative rather than exudative inflammations; therefore, it is difficult to differentiate between granulomas and neoplasms, especially those of mesenchymal origin. Moreover, proliferation of granulation tissue in chronic inflammations confuses cytopathologists because mesenchymal cells (e.g., fibroblasts) are generally in the process of proliferation. In these cases, a definitive diagnosis must be made by histopathological examination.

**Conclusion**

Cytologic examinations are very useful and are becoming an essential tool in clinical veterinary medicine. However, there are some pitfalls caused by certain features of cytologic examination. Therefore, we should understand the advantages and disadvantages of these procedures before performing them.
Pathology of Hepadna Virus Infection in Humans and Animals

K. Abe

Department of Pathology, National Institute of Infectious Diseases, Tokyo, Japan
Department of Pathology, Harbin Medical University, Harbin, China, kenjiabe@nih.go.jp

Keywords: animal, Hepadna virus, human

Hepatitis B virus (HBV) is a DNA virus belonging to the Hepadnaviridae family, in which two separate genera covering mammalian (Orthohepadnavirus) and avian hepadnaviruses (Avihepadnavirus) have been proposed (Table 1).

Human HBV infection is a global health problem. HBV is a bloodborne pathogen and prevalent in Asia, Africa, southern Europe and Latin America. The biggest issue of HBV infection is associated with a 100-fold increase in risk for hepatocellular carcinoma (HCC) development relative to non-carriers. Thus, HBV is one of the most important risk factors in human cancer epidemiology.

1. Human HBV

Genotype and its geographic distribution: HBV is now divided into at least nine genotypes, named A to I (Fig. 1). These HBV genotypes can be further subdivided into subgenotypes. Interestingly, the major Asian strain of HBV consisting of genotypes B and C has strong genetic variation and forms various subgenotypes.

The prevalence and distribution of HBV genotypes vary geographically (Table 2). Genotype A is found in northern Europe, North America and Africa. Genotypes B and C are characteristic of Asia and Oceania, whereas genotype D has a worldwide distribution, predominating in the Mediterranean area. Genotype E is found in Africans on the West coast of Africa and Madagascar on the East; genotype F is identified mostly in the regions of South America; and genotype H is confined mainly to the Amerindian populations of Central America and also has been found in California and Japan. Genotype G has been limited to HBV carriers in France, Germany, United Kingdom, Italy and the U.S.A. Recently identified new genotype I is found only in Vietnam and Laos so far (1,2).

Relationship between genotypes and disease progression:
The course of HBV infection can be affected by a number of factors, such as the age of acquisition and the route of the infection; the immune competence of the host; the influence of environmental factors such as alcohol intake, iron overload and exposure to aflatoxin; and the most important factor, HBV variability: genotypes and mutations.

The effect of genotype on disease progression has been investigated in numerous studies, especially for areas where HBV is endemic and genotypes B and C prevail. For example, liver dysfunction was observed less frequently in hepatitis B carriers with the adw serological subtype (mainly genotype B) compared to those with the adr serological subtype (mainly genotype C). Seroconversion from HBeAg to anti-HBe positivity occurs much earlier in genotype B than genotype C carriers. Fibrosis or cirrhosis was found more frequently, with more severe histological damage, in genotype C than in genotype B.

However, the relationship between HBV variability and disease progression is still controversial. Genotype D was associated with more severe liver disease and HCC in young patients in India, but this result was not confirmed by another study in the same country. Similarly, genotype B in Japan was associated with development of HCC at older age whereas the mean age of HCC patients infected with genotype B in Taiwan and China was significantly younger than those infected with genotype C. These differences have been attributed to be an outcome of host factors, the intake of aflatoxin, and more importantly, the virological variability of HBV, such as the genotypes, subgenotypes, mutations, and geographical regions where the studies have been conducted. The attributes of the genotypes may account not only for differences in the prevalence of HBV mutations in various geographic regions, but may also be responsible for differences in the clinical outcome and response to antiviral treatment.

Recombination, mutations and pathogenesis: HBV genome evolves with an estimated rate of nucleotide substitution at 1.4-3.2x10^-5/site per year (3). Although the S gene of HBV is a useful and adequate target for genotype identification, the complete genomic sequence of HBV provides additional information concerning phylogenetic relatedness and detection of inter- or intra- genotype recombination. To date, inter-genotype recombination with genotype B/C, C/D, A/D, A/G, A/E and A/C/G has been reported. The recombination between different genotypes could play an important process in the evolution and genesis of new classification of HBV.

Due to the absence of the proof reading function of DNA polymerase, the HBV replication process has higher mutation rate than that of other DNA viruses. Although mutations can occur randomly along the HBV genome, the overlapping ORFs of HBV limit the number and location of viable mutants. Naturally occurring mutations of HBV have been described in all four genes, but are more fully characterized in the pre-S/S and core promoter/pre-core regions.

The pre-S1, pre-S2 and S genes encode for the envelope proteins of HBV. The pre-S region has been proven to mediate hepatocyte attachment of the virus. This region also has B cell and T cell epitopes and carries the S promoter site for controlling the production of middle and major S proteins. These findings suggest mutations in this region could have an important role in the pathogenesis of HBV. Expression of HBV proteins may have
Fig. 1 Phylogenetic tree constructed on the full length genome of HBV representing classification of nine genotypes and related subgenotypes of HBV. Asian strain of HBV has genetic variation.

Fig. 2 Comparison of HBV and WHV virions in serum.

Fig. 3 Hepatocellular carcinoma in a woodchuck infected with WHV

...a direct effect on cellular functions, and some of these gene products may favor malignant transformation. Cross-sectional studies demonstrated that the presence of pre-S mutants in serum and liver has been found to carry a high risk for the development of HCC in patients with chronic HBV infection. Importantly, pre-S mutants could initiate endoplasmic reticulum stress-dependent signals to induce oxidative DNA damage necessary in carcinogenesis (4, 5). Moreover, transgenic mice harboring pre-S2 mutant developed nodular liver cell dysplasia and HCC (6). HBV pre-S mutants, particularly the pre-S2 mutants, are now recognized as viral oncoproteins of HBV-related HCC. Our recent study showed deletions at nt 4-54 in the pre-S2 region of HBV are hot spot region of mutation and such mutant could have an important role in hepatocarcinogenesis in childhood HCC (7).
2. Animal hepadna viruses

Hepadna viruses have been isolated from several species of birds and rodents including woodchuck hepatitis virus (WHV), ground squirrel hepatitis virus (GSHV), and arctic squirrel hepatitis virus (ASHV). New hepadna virus was isolated from woolly monkeys, a New World primate. Woolly monkey HBV (WMHBV) is the first nonhuman primate hepadna virus. And now, nonhuman primate HBV has been reported from a wide range of nonhuman primates including chimpanzee, gorilla, orangutan, and gibbon. The subsequent isolation of unique HBV genome sequences from these nonhuman primates suggests the existence of HBV strains indigenous to these animals. Liver pathology in infected primates is poorly understood. So far, there was no report of HCC in nonhuman primate HBV-infected animals.

WHV isolated from woodchucks was the first of the mammalian and avian hepadna viruses described after discovery of the human HBV (Fig. 2) (8). Woodchucks chronically infected with WHV develop progressively chronic hepatitis and HCC (but not accompanied with cirrhosis), which present as lesions that are remarkably similar to those associated with HBV infection in humans (Fig. 3). In WHV-infected woodchucks, foci of altered hepatocytes (FAH) appearing in non-cancerous liver parenchyma are characteristic. This FAH is identical to those caused by chemical hepatocarcinogens in rats and mice, suggesting pre-neoplastic lesions. Metastasis of HCC outside the liver is very rare. Integration of WHV DNA into or near N-myc family of proto-oncogenes could play a role in enhancing genomic instability and may trigger specific oncogenic pathways. In woodchuck HCCs, pre-S mutants were identified in 62.5% (5/8) and of exclusively pre-S1 deletion. Chronic WHV carrier woodchucks have become a valuable animal model for the preclinical evaluation of antiviral therapy for human HBV infection. Ground squirrels and arctic squirrels infected with GSHV and ASHV, respectively, also display an increased prevalence of HCC.

References

Table 1. Common characteristics of hepadna viruses

<table>
<thead>
<tr>
<th>Feature</th>
</tr>
</thead>
<tbody>
<tr>
<td>● Hepatotrophic</td>
</tr>
<tr>
<td>● Small circular DNA genome</td>
</tr>
<tr>
<td>3-3.3 kb pair in size</td>
</tr>
<tr>
<td>Single-stranded region of fixed polarity and variable length</td>
</tr>
<tr>
<td>● Virion-associated DNA polymerase activity</td>
</tr>
<tr>
<td>● Persistent infection</td>
</tr>
</tbody>
</table>

Table 2 HBV subgenotype and its geographical distribution

<table>
<thead>
<tr>
<th>Subgenotype</th>
<th>Subtype</th>
<th>Genome length (nt)</th>
<th>Geographical distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>adw2</td>
<td>3221</td>
<td>South Africa, Sub-Saharan Africa, Malawi, Philippines</td>
</tr>
<tr>
<td>A2</td>
<td>adw2</td>
<td>3215</td>
<td>Europe, USA, Russia</td>
</tr>
<tr>
<td>A3</td>
<td>ayw1</td>
<td>3215</td>
<td>West/Central Africa, Cameroon, Gambia</td>
</tr>
<tr>
<td>A4</td>
<td>ayw1</td>
<td>3215</td>
<td>Malawi, Gambia</td>
</tr>
<tr>
<td>A5</td>
<td>adw2</td>
<td>3215</td>
<td>Nigeria</td>
</tr>
<tr>
<td>B1*</td>
<td>adw2</td>
<td>3215</td>
<td>Japan, Korea, Alaska, Greenland</td>
</tr>
<tr>
<td>B2*</td>
<td>adw2</td>
<td>3215</td>
<td>China, Hong Kong, Thailand, Laos, Vietnam</td>
</tr>
<tr>
<td>B3*</td>
<td>adw1</td>
<td>3215</td>
<td>Indonesia</td>
</tr>
<tr>
<td>B4*</td>
<td>ayw1</td>
<td>3215</td>
<td>Vietnam, Cambodia, Laos</td>
</tr>
<tr>
<td>B5*</td>
<td>ayw1</td>
<td>3215</td>
<td>Philippines</td>
</tr>
<tr>
<td>B6</td>
<td>adw2</td>
<td>3215</td>
<td>Canada, Alaska</td>
</tr>
<tr>
<td>C1*</td>
<td>adw2</td>
<td>3215</td>
<td>Vietnam, Cambodia, Laos, Thailand, Myanmar, South China</td>
</tr>
<tr>
<td>C2*</td>
<td>adw2</td>
<td>3215</td>
<td>Japan, Korea, North China</td>
</tr>
<tr>
<td>C3*</td>
<td>adwr</td>
<td>3215</td>
<td>Polynesia</td>
</tr>
<tr>
<td>C4*</td>
<td>ayw3</td>
<td>3215</td>
<td>Australian aborigine</td>
</tr>
<tr>
<td>C5*</td>
<td>adw2</td>
<td>3215</td>
<td>Philippines, Indonesia</td>
</tr>
<tr>
<td>D1</td>
<td>ayw2</td>
<td>3215</td>
<td>Middle Asia, India, Uzbekistan, Turkey, Iran</td>
</tr>
<tr>
<td>D2</td>
<td>ayw3</td>
<td>3215</td>
<td>India, Nepal, Russia, Estonia</td>
</tr>
<tr>
<td>D3</td>
<td>ayw2</td>
<td>3215</td>
<td>Mediterranean area, South Africa</td>
</tr>
<tr>
<td>D4</td>
<td>ayw2</td>
<td>3215</td>
<td>Australia, Papua New Guinea</td>
</tr>
<tr>
<td>D5</td>
<td>ayw2</td>
<td>3215</td>
<td>India</td>
</tr>
<tr>
<td>E</td>
<td>ayw4</td>
<td>3215</td>
<td>West Africa, Madagascar, Angola, Brazil</td>
</tr>
<tr>
<td>F1</td>
<td>adw4q-</td>
<td>3215</td>
<td>Central America, Argentina, Venezuela</td>
</tr>
<tr>
<td>F2</td>
<td>adw4q-</td>
<td>3215</td>
<td>Central America, Argentina, Brazil</td>
</tr>
<tr>
<td>F3</td>
<td>adw4q-</td>
<td>3215</td>
<td>Colombia, Venezuela, Panama</td>
</tr>
<tr>
<td>F4</td>
<td>adw4q-</td>
<td>3215</td>
<td>Bolivia, Argentina, Venezuela</td>
</tr>
<tr>
<td>G</td>
<td>adw2</td>
<td>3215</td>
<td>USA, France</td>
</tr>
<tr>
<td>H</td>
<td>adw4</td>
<td>3215</td>
<td>Nicaragua, Mexico, USA (California)</td>
</tr>
<tr>
<td>I1*</td>
<td>adw</td>
<td>3215</td>
<td>Vietnam, Laos</td>
</tr>
<tr>
<td>I2*</td>
<td>ayw</td>
<td>3215</td>
<td>Laos</td>
</tr>
</tbody>
</table>

*Asian strain
Suppression of Rabies Virus Propagation in Mice Brain by Intracerebral Immunization of Inactivated Virus

Y. Sunden, S. Yano, K. Ochiai, T. Umemura*

Lab. of Comparative Pathology, Dept. of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University *corresponding author: utakashi13@hotmail.com

Keywords: Intracerebral, mice brain, Rabies virus

Introduction

Rabies is one of the classical viral zoonoses and lethal in many mammals including humans. The virus replicates in the central nervous system (CNS) and no effective treatment is available in rabid animals so far. In our previous studies, we demonstrated the modes of transneural spread of viruses including influenza virus by in vivo and in vitro studies (1-3) and also exhibited an efficacy of intrathecal immunization (direct inoculation of antigens into cerebrospinal fluid to elicite immunological reaction within the CNS for the prevention of transneural invasion of the viruses to the CNS) (Table 1) (4-6). The object of this study is to examine the efficacy of intracerebral (IC) immunization, one of the intrathecal immunizations, against rabies virus propagation in mice brain.

Material and Methods

Mice were immunized with inactivated rabies viruses via subcutaneous (SC) or IC route, and lethal doses of live rabies virus, CVS strain, was inoculated into the brain of immunized mice. Clinical signs and body weights of mice were recorded. Measurement of antibody titers in blood, protein expression and histopathological analysis of brain were performed.

Results and Discussion

Progressive paralytic neurological signs were observed in all control mice and 75% of SC immunized mice, whereas in only 20% of IC immunized mice. The neutralizing antibody titer in blood plasma was significantly elevated in both SC and IC immunized group. Analysis of whole brain lysate of each mice showed that total immunoglobulin proteins were highly induced in IC immunized mice and they had virus neutralizing abilities. Histopathological examination of brain revealed severe encephalitis and disseminated virus antigens including nerve processes in control mice, but not in IC immunized mice.

It was clearly shown that IC immunized mice could induce preventive immune-response against intracerebrally inoculated rabies virus. Both systemic and intracerebral immune-responses are now under investigating which contribute to suppressing the virus propagation in mice CNS.

References

Clinical Trials of Adipose Derived Stem Cells and Muscle Derived Stem Cells

I. H. Hong, M. R. Ki, J. K. Park, A. R. Ji, S. I. Park, K. S. Jeong*

Department of Pathology, College of Veterinary Medicine, Kyungpook National University, Daegu 702-701, Republic of Korea
*corresponding author: jeongks@knu.ac.kr

Keywords: adipose, muscle, stem cells

Introduction
Adipose derived stem cell (ADSC) and muscle derived stem cells are strong promising cell therapy for diverse type of both human and animal patients such as plastic, reconstructive surgery and disease models, but information of diverse clinical therapy and guidelines for cell therapy is not established due to the very limited clinical trials.

Material and Methods
Here the authors have been tried for diverse cases such as autoimmune skin disease, muscle weakness, systemic ageing process, chronic renal failure, liver injury and osteoarthritis by using myogenic, adipogenic, cartilage, hepatic parenchymal progenitor cells, which was established in our laboratory. The stem cell extraction techniques likely adipose derive stem cells and muscle derived stem cells quite well explained for multipotent stem cells which has the ability to differentiate into several mesodermal lineages cell population. After primary cultivation passages 3 and stem cells expansions around 1x10^6 cells via different injection sites have been tried for patients and in vitro signaling experiments at this point.

Results and Discussion
Clinical data after stem cell therapy was successfully satisfied with their host and also animals have shown promising therapeutic effects. In case of canine renal failure, stem cell therapy improved renal failure via evidence of biochemical analysis and mobility without any side effects, and also showed therapeutic effects on osteoarthritis of canine via evidence of biochemical analysis for specific markers of arthritis and increase mobility of all joint, and autoimmune skin disorders via analysis of histopathology scoring system. In addition, stem cell transplantation and therapy of experimental liver injury and muscle injury models can be successfully established in our laboratory and improve liver function and muscle function too.

All these data taken together suggest that both adult stem cell therapy for canine patients and experimental studies are promising treatment for diverse clinical cases at this point.
Cytological Diagnosis of Neoplasia

D.J. Meuten*, M.A. Thrall

*President, ACVP, 1Department of Microbiology, Immunology and Pathology, College of Veterinary Medicine & Biomedical Sciences, Colorado State University, USA

Keywords: cytology, diagnosis, neoplasia

Several preliminary considerations are important in the cytologic diagnosis of neoplasia. Information regarding the location/size of the lesion and the method of specimen collection are important in deciding if the specimen is representative of the lesion. Key to this diagnosis is to determine that the “lesion” is not inflammatory and is not a normal structure. The more neutrophils you see, the more the lesion is inflammatory and not a tumor. True, neutrophils can infiltrate tumors and be present, but your thumb-rule is still the same — the greater the inflammation, the less likely there is a neoplasm. Another general rule is: Every time you get ready to diagnose a tumor with inflammation consider a granulomatous lesion as the other differential. The large cells you are seeing that you think are neoplastic may be macrophages. Macrophages in tissues can be large and appear epithelial in fact these types of macrophages are referred to as “epithelioid macrophages”. If some of these cells in question have vacuoles of various sizes, and have phagocytosed something, they are probably macrophages and not tumor cells. When there are numerous mononuclear cells with minimal or no inflammation, then it is usually one of the following possibilities: a tumor, hyperplasia, or a normal structure. Normal structures generally yield few cells vs. numerous cells exfoliate from a tumor (usually). The more uniform the mononuclear cells, the more likely it is a normal structure, or at least “benign. This is usually not a problem; most of us do not go around aspirating normal structures! The cytologic features of the cells are evaluated to classify the tumor as benign or malignant and as to its tissue type, i.e., epithelial, mesenchymal, or round cell. An attempt can be made to assess the potential malignancy of the tumor by identifying cytologic criteria of malignancy (discussed below), the most important of which is variability. If the cells are numerous, but resemble a normal tissue/organ, their appearance must be critically assessed to decide between normal (perhaps the lesion was missed), benign proliferation (focal hyperplasia) or a benign tumor. This is really only a dilemma when aspirating normal structures. For dermal lesions forget hyperplasia and normal structure, you are aspirating a lump and it is not normal. Decide this: is the dermal lesion inflammatory or not inflammatory. If it’s not inflammatory then decide if it is a round cell, mesenchymal or epithelial cell tumor, then try to decide if it is benign or malignant.

Determincation of cell type: The shape of the cells and their nuclei, and the presence or absence of cohesive tendencies are the main criteria used in attempting to classify the cell type of a neoplasm. Frequently, the cytologic diagnosis cannot be highly specific, but classification as to epithelial, mesenchymal, or round cell origin and the biologic behavior of the tumor can usually be accomplished. Three of the best criteria to determine origin of a tumor is location, location and location! Example: if you aspirate a mass in the urinary bladder and you recognize neoplastic cells then it is a transitional cell carcinoma until proven otherwise. If you see neoplastic cells in a mass in the distal radius it is an osteosarcoma until proven otherwise. Epithelial cells usually exfoliate easily (numerous cells seen) and tend to be shed in clusters. Cell shape may reflect that of the specific epithelial type (squamous, cuboidal, columnar), but these characteristics are often lost in poorly differentiated tumors. A single large cytoplasmic-vacuole, marked distension of cytoplasm, and/or acinar-like formations (balls, morulae) indicate a glandular epithelial origin. Cells tend to be roundish, polyhedral, large and nuclei tend to be round.

Mesenchymal (connective tissue) cells tend to exfoliate poorly (low cell numbers) and are more prone to exfoliate individually (though poorly organized groups of cells may be closely apposed in highly cellular specimens). Cell shape tends to be more elongated (spindle shaped) and nuclei may be oval or elongated. More specific characterization as to cell type may not be possible unless evidence of some associated cell product can be found. Extracellular matrix material can provide a clue in myxosarcomas, osteosarcomas, and chondrosarcomas.

Identification of intracellular pigment commonly allows a specific diagnosis of melanocytic tumors. If intracellular pigment is seen, the diagnosis is usually easy to make. Purple granules are characteristic of mast cells and black or greenish granules are features of melanomas (described later in notes).

Round cell tumors also exfoliate discrete cells, usually lots of them, but they lack the elongate shape common among connective tissue cells and they don’t form balls or morulae like epithelial tumors. Lymphoma, mast cell tumor, histiocytoma, transmissible venereal tumor, and plasmacytoma are in this group. Specimens from these tumors tend to be highly cellular, and characteristic morphologic features often allow specific cytologic diagnosis (see section on cytology of the skin). Round cells may lie close together on a slide and this is often misinterpreted as an epithelial cluster. Basal cell tumors are epithelial but they may look like a round cell tumor if you do not
appreciate how cells are arranged in rows or in morulae.

**Cytologic criteria of malignancy:** Once a diagnosis of neoplasia is made, the next steps are:

1. Is it epithelial, mesenchymal or round cell? and, more importantly;
2. Is it benign or malignant?

The most useful cytologic features of malignancy can be grouped as follows: 1) general, 2) nuclear.

**General features of malignancy include:** overall high cellularity of the specimen; and heterogeneity or variability of cell features (i.e., generally large, but variably sized and shaped cells and nuclei). As mentioned before, recognition that a population is foreign to the tissue in question is important. The single-most important feature of malignancy is **VARIABILITY.** Variable numbers of nuclei and nucleoli. **Variable sizes of cells, nuclei, and nucleoli.** Variable shape of cells, nuclei, and nucleoli.

**KEY = VARIABILITY OF CELL FEATURES = Size, Shape, Numbers = MALIGNANCY**

**Nuclear features of malignancy include:** high/variable nuclear to cytoplasmic ratio; variation in nuclear size and shape; abnormal multinucleation (i.e., variably sized within same cell); variable number of nuclei/cell; prominent nucleoli of varying size, shape, and number; abnormal mitotic figures; and irregular and/or coarse chromatin pattern. No one feature can be singled out as being of primary importance, but, in general, the nuclear changes are most useful. Highly anaplastic tumors may display many of these abnormalities simultaneously. The cytologic diagnosis in such cases is relatively easy and very reliable. In general, at least three or four criteria should be prominent in a high proportion of the cells in question before a diagnosis of malignancy is made. The more uniform the cells and nuclei, the more likely the tumor is benign. Most of the round cell tumors are benign and look benign. An obvious exception to both of these rules is lymphoma. These tumors are uniform, monomorphic, round, but malignant. These features are diagnostically significant when present, but their absence does not exclude the possibility of malignancy. Not all malignant tumors are cytologically anaplastic, and some malignant tumors are “cytologically” benign e.g., canine thyroid carcinoma, “insulinoma” and canine anal sac adenocarcinoma all may look benign but behave in a malignant fashion.

The presence in a smear of both an inflammatory response and a dysplastic population of tissue cells is a sign to proceed with caution. It brings into question the basic character of the lesion, and complicates the assessment of potential malignancy. Granulomatous inflammation and even pyogranulomatous inflammation versus neoplasia can still trick me!! They can look a lot like neoplasia! This dilemma happens frequently in neoplasms of the urinary system because it is lined by transitional epithelium, which can be quite “dysplastic” even with just inflammation. Histologic assessment will probably be needed in these situations. With histology, the architectural features such as invasion into surrounding tissue, lymphatics and/or blood vessels can be used to help identify malignancy.

"Remember = The most important criterion of malignancy = VARIABILITY!"

**CYTOLOGIC CRITERIA FOR MALIGNANCY**
1. Pleomorphic population of mononuclear cells with few or no inflammatory cells.
2. Large cells (try to find an inflammatory cell for a size reference).
3. Variation in size and shape of nuclei and cytoplasm.
4. High nuclei-cytoplasmic ratio-large nuclei.
5. Nuclear abnormalities
   - Multiple nuclei, varying numbers
   - Large
   - Variation in size and shape of nuclei
   - Lobulation, cleaving, molding, angulation
   - Irregular clumping and dark chromatin
6. Nucleolar abnormalities-different numbers, sizes, and shapes per nucleus.

**Hyperplasia vs. neoplasia** can be difficult to assess cytologically. Each shares similar cytologic and subcellular events (i.e., dark blue cytoplasm for both is due to increased RNA, large nuclei, chromatin clumping, nucleoli, and mitotic figures are all features of young cells. The key is variability. Malignant cells have many of the same features seen in hyperplastic cells, but these features are heterogenous in malignant cells, e.g., variably sized and shaped cytoplasm, nuclei, and nucleoli.
Introduction to the Principles and Practice of Veterinary Surgical Pathology

P.C. Stromberg

Department of Veterinary Biosciences, The Ohio State University, USA

Corresponding author: Stromberg.1@osu.edu

Keywords: biopsy, pathology, surgical

Surgical pathology is a transient partnership between a clinician and a pathologist on behalf of the patient to aid the clinician in 1) making or confirming a diagnosis 2) assisting in prognosis by assessing the progress of therapy, grading and staging tumors and determining the completeness of excision. It is fundamentally different than an autopsy in that the task is performed by 2 different veterinarians. To obtain the best result for the patient, the clinician taking the biopsy and the pathologist reading it must understand the problems that both face in the process. The biggest and most persistent problem in veterinary surgical pathology today is the poor communication between clinicians and pathologists. Much of the quality of the outcome depends on clinicians who desire more from the pathologist without understanding the importance of their input. The rules that govern medical practice apply to surgical biopsy. The First Rule of Medicine is “Above all do no Harm”. The First Rule of Surgical Pathology is “Don’t violate the first rule of medicine” then “Get a diagnostic biopsy”.

What is a Diagnostic Biopsy: A diagnostic biopsy is an
1) adequate amount of tissue
2) representative of the pathologic process
3) sufficiently free of artifacts to permit a definitive evaluation accompanied by
4) a signalment, history and description of the lesions and
5) the clinicians differential diagnosis, rule outs or thoughts. Total Patient Evaluation

An Adequate Amount of Tissue: Clinicians have a wide variety of techniques for tissue collection but the trend in veterinary medicine today is toward small specimens which pose significant barriers to acquiring a diagnostic biopsy. Fine needle aspirates (FNA), Tru-cut or “EZ core” biopsies, punch specimens, endoscopic and laparoscopic samples all procure very small amounts of tissue that may not be diagnostic. Incision and excisional biopsies provide more (or all) of the lesion which may be adequate but have their own problems and may not be possible given the clinical conditions.

Representative of the Pathologic Process: Because the pathologic process may not be uniformly distributed in the tissue or organ, a small biopsy may not contain the diagnostic tissue. Likewise, inflammation, necrosis, and reactive tissue may obscure the lesion in small samples.

Sufficiently Free of Artifacts: Artifacts are a structure or substance not normally present in the tissue but produced by some external agency or action. Crush artifacts are common in small specimens and fixation artifacts are common in large specimens, both of which can produce a poor quality specimen unsuitable for diagnosis. Both of these problems are the responsibility of the clinician taking the biopsy. Fixation artifact can be eliminated by 2 simple principles. 1) Place no tissue in fixative that is thicker than 0.5-1.0cm and 2) make sure the amount of 10% NBF fixative is at least 10-15X the volume of the tissue place in it. That means that large samples should be Bologna sliced, trimmed to the right size and placed in biopsy jars to satisfy the principles of biopsy. Size is the most important variable in getting a diagnostic biopsy because of the problems with crush and fixation artifact. Small samples are easily destroyed by crush artifact and large specimens risk poor fixation or inadequate sub sampling by the clinician resulting in the submission of tissue that does not contain the diagnostic pathologic process.

Accompanied by a Signalment, History and Description of the Lesions: The communication principle for clinicians is “Help the Pathologist to Help You”. Because the pathologist was not involved in the biopsy procedure and usually knows little about the patient or the clinical problem, it is imperative that the clinician share with the pathologist all of the essential clinical aspects of the case to orient the pathologist or “Frame” the case. Just as clinicians practice the principle of “Total Patient Evaluation”, so should pathologists but their ability to do so depends almost entirely on the clinician to provide the information needed. If he or she does not, there is a risk that the pathologist will not make the correct diagnosis and the best interests of the patient will not served. In this
regard, a properly designed biopsy submission form is essential for reminding clinicians of the importance of this information and the value of their supplying it. Good submission forms are designed so that the clinician can tell the pathologist

1) What they saw
2) What they did
3) What they think and
4) What they want.

The 5 important information elements of the submission form are a

1) Signalment
2) Clinical or historical data pertinent to the case
3) Precise location of the lesion
4) Descriptive characters of the lesion that was sampled such as size, color, extent, shape and distribution and the
5) Clinicianís thoughts such as the differential diagnosis, what rule outs they are interested in, why they collected the biopsy and what they want the pathologist to do.

Communication Principles for the Surgical Pathologist: “Give the Clinicians What They Need to Manage the Case”. Answer the question, “Why did the clinician take a biopsy”? Usually the biopsy was taken to get a diagnosis or confirm a presumptive diagnosis; secondly to assist in prognosis. This may be to determine if the lesion has been completely excised or to characterize behavioral aspects of the pathologic process that would help the clinician predict the clinical course. This is becoming the biggest challenge in veterinary surgical pathology since clinicians want to get more out of the procedure to justify the cost. Is the tumor completely excised? Is there evidence of local tissue invasion or vascular invasion that would predict metastasis or recurrence of the pathologic process? Increasingly it may be to grade tumors especially mast cell tumors and soft tissue sarcomas. Although pathologists should always provide a morphologic diagnosis, which is a phrase or one lime summary of the primary, characteristic or most important pathologic processes present in the biopsy, he or she should strive to provide a specific clinical disease diagnosis whenever possible. This is the translation of the lesions observed in the biopsy into the name of a clinical disease condition. This is the optimal outcome of the biopsy because it places the pathologic process into the clinicians world of clinical medicine and makes more likely that the clinician will apply the correct therapy. However, for pathologists to consistently make the diagnosis of a clinical disease they must be able to perform a total patient evaluation which usually depends on the clinician supplying the important information on the submission form.

When the results of the biopsy report do not make sense to the clinician, he or she should phone or email the pathologist and ask for clarification. Such phone conversations should be welcome by surgical pathologists because they provide the opportunity for “teaching moments” that can be extremely valuable to both parties. Clinicians occasionally desire the opinion of another pathologist. This is a standard practice and no pathologist should be offended by the request. Pathologists should readily agree to have their diagnoses reviewed and to perform reviews of other pathologistís diagnoses. It is an opportunity for both pathologists AND clinicians to learn. There is evidence that clinicians do not fully understand the subjective nature of pattern recognition in diagnostic pathology and how two pathologists can look at the same slide and make 2 different diagnoses.

Everyone involved in the process of surgical biopsy should understand the limitations of the procedures and have reasonable expectations for what can be accomplished. A surgical biopsy is NOT another laboratory test you order like a hemogram i.e. it is not an objective measurement of discrete variables recorded by an instrument and reported in metric units. There is much art as science in the biopsy interpretation with the pattern of pathologic processes often overlapping. It often requires considerable human judgment which is extremely difficult in the practice of medicine. If both clinicians and pathologists attend to the important aspects of this task that they are responsible for, they will raise the likelihood or obtaining the correct and complete diagnosis that the clinician needs to give aid to the patient. That is after all the purpose of the biopsy.

The Yin and Yang symbol which represents the ancient Chinese understanding of how things work in nature is relevant to veterinary surgical biopsy. The symbol embodies the continual dynamic tension in the surgical pathology arena between clinicians and pathologists in meeting the needs of the patient all balanced by the First Rule of Medicine and the First Rule of Surgical Pathology.
Mammalian Models for Studies of Transmission of Highly Pathogenic Avian Influenza A (H5N1) Viruses with Meat from Infected Poultry

Y.K. Kwon1*, M.S. Kang1, M.I. Kang2, A.S. Lipatov3, D.E. Swayne3

1National Veterinary Research & Quarantine Service, 480, Anyang city, S. Korea, 2Veterinary Medical Science, Chonnam National University, S. Korea, 3Southeast Poultry Research Laboratory, ARS, USDA, USA,

*Corresponding author: kwonyk@nvrqs.go.kr

Keywords: animals, comparative pathology, H5N1, HPAI infection

Influenza viruses are negative sense, single stranded, segmented RNA viruses belonging to the family Orthomyxoviridae (1). Whereas influenza virus types B and C are mainly human pathogens, influenza A viruses act as pathogens in many mammalian species including humans and in birds. Influenza A viruses are classified into distinct subtypes according to different haemagglutinin and neuramindase glycoprotein molecules expressed on the surface. In avian influenza viruses, all different subtypes described until today are found. They can typically produce syndromes ranging from asymptomatic infection to respiratory disease and drops in egg production to severe, systemic disease with near 100% mortality (2). The latter form of disease is the results of infection by high pathogenicity or highly pathogenic avian influenza (HPAI) viruses. HPAI virus subtype H5N1 was first detected in 1996 in domestic geese in China. After several reassortment events, this avian virus not only caused serious disease in poultry and wild birds, but also crossed the species barrier infecting people in Hong Kong in 1997 (3). Mammalian species known to be susceptible to HPAI virus subtype H5N1 are humans, ferrets, dogs, mice, stone martens, cynomolgus monkeys, civets, domestic cats, tigers and leopards. (4, 5)

To date, human to human transmission of H5N1 HPAI viruses has been very limited, and most cases of infection in humans have occurred through close contact with infected live or dead poultry. A few cases in humans have implicated direct oral contact either through consumption of uncooked blood from infected ducks and raw poultry products or through oral exposure from sucking exudate from the upper respiratory tract of infected fighting cocks (6, 7). We tried to test whether animal models (ferrets) could be infected through oral consumption or gastric gavage of infected chicken meat, which were very similar environmental conditions of human infection routes. As a result, consumption of infected meat by ferrets resulted in both severe respiratory and systemic infection with predominant involvement of the liver, pancreas, and small intestine (due to A/Vietnam/1203/04 virus). Also direct intragastric exposure to infected meat resulted in lethal systemic disease mainly affecting the intestine, liver, and pancreas but not involving the lungs. Using another animal model (mice), A/Whooper swan/Mongolia/244/05, was able to infect mice after intragastric inoculation in liquid, whereas no evidence of infection was observed in ferrets after intragastric inoculation. In brief conclusions, exposure of the digestive system to H5N1 influenza viruses could initiate infection through intestinal infection with spread to the liver and pancreas (4).

Until today, there have not been published official reports that HPAIV (H5N1) causes natural infections in the domestic pigs with clinical signs. However, Domestic pigs which are susceptible to infection with both human and avian influenza A viruses are one of the natural hosts where genetic reassortment events could occur. To estimate pathogenicity of H5N1 viruses in the pigs, we infected pigs with four H5N1 viruses representing clades 1 and 2, and subclades 2.1, 2.2 and 2.3 (A/Vietnam/1203/04, A/Chicken/Indonesia/7/03, A/Whooper swan /Mongolia/244/05, and A/Muscovy
Histological features of H5N1 infection in pigs were characterized and compared with those caused by swine H3N2 and H1N1 viruses. In the pigs infected with H5N1 viruses, mild to moderate bronchitis and alveolitis were found on day 5 post inoculation. The lung lesions included moderate lymphocytic infiltration around peribronchiolar and perivascular areas, mild degeneration to necrosis of bronchiolar epithelium, and moderate necrotic cell debris in the airways of bronchioles and alveoli. Immunohistochemically, viral antigen was detected in bronchiolar epithelium. On day 14 days post-inoculation, there were no histological lesions in any visceral organs including lungs. By comparison, the respiratory lesions from pigs infected with swine virus (H3N2) were more severe and more extensive than those from pigs infected with H5N1 viruses. The lungs from pigs infected with swine viruses on 5 days had severe bronchopneumonia characterized by severe degeneration and necrosis of bronchial epithelium and accumulation of necrotic cellular debris within airway lumens. In addition, the nasal cavities of pigs infected with H3N2 swine virus showed mild vacuolar degeneration and necrosis of mucosal epithelium. Mild lymphocytic infiltration around peribronchial areas was still evident in the lungs of swine viruses-infected pigs on days 14 days post-inoculation. However, no viral antigen was detected to any tissues or organs. In addition, piglets in one group of 4 were fed breast and thigh meat from chickens that died from infection with A/Whooper swan/Monglia/05 H5N1 virus. The meat was chopped into small pieces approximately 4 cm x 2 cm x 0.5 cm in size. No disease signs such as significant weight loss, changes in food consumption were observed in exposed pigs during the 14 day observation period. Virus was detected in nasal swabs from 2 of 4 pigs on day 3 only. No virus was detected in rectal swabs. However, virus-neutralizing antibodies to the virus were detected in serum samples from pigs collected on day 14 after consumption. Those results suggested that consumption of raw or uncooked poultry meat contaminated with avian influenza virus can transmit the virus to pigs and mammals (8, 9).

References
8. Lipatov et al., 2008. PLoS. Pathog. 4: e1000102
Acute Necrotizing Hepatitis due to *Francisella tularensis* subsp. *holarctica* Infection

C. H. Park

Department of Veterinary Pathology, School of Veterinary Medicine, Kitasato University, Towada, 034-8628 Japan,
E-mail: baku@vmas.kitasato-u.ac.jp

**Keywords:** necrotizing hepatitis, hare, *Francisella tularensis*

**Clinical History and Gross Findings**
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.

At necropsy, marked enlargement of the spleen (10x2x1 cm), enlarged cervical lymph nodes (1.5x 1x0.5 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 (0.7x0.7x0.5 cm) was present in the right anterior lobe. The pulmonary lymph nodes were mildly swollen.

**Histological Diagnosis**
Acute necrotizing hepatitis due to *Francisella tularensis* subsp. *holarctica* infection

**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 *F. 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.

References
Hemophagocytic Histiocytic Sarcoma in a Cow

K. Matsuda

Department of Veterinary Pathology, School of Veterinary Medicine, Rakuno Gakuen University
582 Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan, email: kmatsuda@rakuno.ac.jp

Keywords: cow, hemophagocytic, histiocytic sarcoma

Clinical History
An 11-year-old Japanese Black cow had decreased feed intake, decreased activity, and an abnormal gait that progressed to staggering and hind-limb paralysis. The cow was anemic and leukocytopenic. Serum biochemistry abnormalities included mild increases in glucose, aspartate aminotransferase, γ-glutamyltransferase, total bilirubin, and a slight decrease in total cholesterol. Seventeen days after the onset of clinical signs, the cow was euthanized.

Diagnosis
Hemophagocytic histiocytic sarcoma

Gross Findings
The spleen was diffusely enlarged to 102x33x8 cm and 11.9 kg. The splenic parenchyma was firm with indistinct white pulp and no discrete masses. The liver was normal in size and had scattered foci of telangiectasis through the parenchyma. The dorsal aspect of the 4th thoracic vertebral body protruded focally into the spinal canal, because of dark-red nodular expansion of the subperiosteal marrow with irregular discontinuity of the associated cortex. Similar, but milder, lesions were in the 2nd, 6th and 7th thoracic vertebrae.

Histopathological Findings
Histologically, the splenic red pulp was congested and expanded by numerous histiocytes. The white pulp was compressed and atrophied. The neoplastic histiocytes had a pleomorphic nucleus with pale eosinophilic cytoplasm. Mitotic rate ranged from 0 to 2 per high-power field. Many had phagocytized 1 to several erythrocytes, which resulted in peripheral displacement of the nucleus.

In the liver and lungs, erythrophagocytic histiocytes were detected in vascular lumina, but not in the extravascular space. Histiocytic proliferation also comprised the vertebral masses and adjacent marrow infiltration. The histiocytes in the vertebrae resembled those in the spleen but had higher nuclear atypia and less erythrophagocytosis. The neoplastic histiocytes only rarely had engulfed granulocytes. Axonal swelling was most prominent in the ventral funiculi of the 4th thoracic spinal cord segment, where the largest vertebral mass was. Central chromatolysis was observed in ventral horn neuronal soma from the 3rd to 4th thoracic segments; macrophages infiltrated dilated myelin sheaths in the ventral funiculi of the cord from the 3rd thoracic to 3rd lumbar segments.

Immunocytochemical Results
Paraffin-embedded sections of the vertebral mass and normal bovine spleen were used. Approximately half the histiocytes, including erythrophagocytic histiocytes, in the vertebral mass expressed CD68, MHC-II, CD18 and lysozyme. In normal bovine spleen, cells positive for CD68 and lysozyme were confirmed to the red pulp, whereas reactivity for MHC-II and CD18 was detected in both red and white pulp. The neoplastic histiocytes were negative for CD3, CD79a, CD20 or lambda light chains.

Discussion
Hemophagocytic histiocytic sarcoma (HS) is a histiocytic proliferation of macrophage origin and is considered a variant of histiocytic sarcoma or malignant histiocytosis. The lesions are characterized by diffuse splenomegaly without distinct mass formation and a proliferation of histiocytes with marked phagocytic activity in the spleen and bone marrow. Neoplastic histiocytes primarily proliferate in the spleen and bone marrow, with subsequent intravascular spread to liver, lungs and lymph nodes; the neoplastic histiocytes have avid phagocytic activity in all sites. In canine and feline hemophagocytic HS, the neoplastic histiocytes are CD11d+/CD18+/MHC-II+ (canine) or CD1c−/CD11b+/CD18+/MHC-II+ (feline), that is characteristic of macrophages of the splenic red pulp and bone marrow in normal animals. Antibodies to bovine CD1c, CD11b, CD11c or CD11d were not available. In normal bovine spleen, however, cells immunohistochemically positive for CD68 and lysozyme were confirmed to the red pulp. This may be useful to distinguish macrophages from dendritic cells, which reside in the white pulp. In the present case, a proportion of the histiocytes with and without erythrophagocytosis in the vertebral mass were immunohistochemically positive for CD68, CD18, lysozyme and MHC-II. These results are consistent with origin from macrophages, rather than dendritic cells. Hind-limb paralysis in this case was attributed to spinal cord compression by the vertebral masses. Formation of discrete masses is unusual with hemophagocytic HS.

References
Iron Intoxication in Ring-tailed Lemurs (*Lemur catta*)

Y.-C. Tsai¹, S.-H. Hsiao¹,²,³, M.-Y. Chia¹, V. F. Pang¹,²,³

¹Division of Pathobiology, School of Veterinary Medicine, National Taiwan University
²Department of Veterinary Medicine, National Taiwan University
³Veterinary Hospital, National Taiwan University *Corresponding author: i_chieh22@yahoo.com.tw

**Keywords:** intoxication, iron, ring-tailed lemur

**Clinical History:** In the two days of 9th and 10th of May in 2005, there was a 100% incidence of mortality (3/3) of ring-tailed lemurs (*Lemur catta*) in the lemur enclosure of tropical rainforest area in Taipei Zoo. On the 9th of May in 2005, two adult ring-tailed lemurs (*Lemur catta*) were found dead lying beside the mineral rock with no apparent abnormalities. Another 2- to 3-year-old ring-tailed lemur displayed clinical syndromes of depression, anorexia, lethargy, anemia, and disorder of coagulation and medication was provided but it died the next day. Members in the same enclosure included 6 ring-tailed lemurs (*Lemur catta*), 8 ruffed lemurs (*Varecia variegata*), and 15 brown lemurs (*Eulemur fulvus*) and the other two species were not affected. The lemurs were fed on fruits, leaves, monkey fodder, vitamins, salt, and high-fiber food as daily consumption.

**Diagnosis:** Iron intoxication

**Gross Findings:** Grossly, 10 to 20 ml of clear yellow, red to dark red thoracic effusion and 3 to 9 ml of clear green-yellow to yellow-brown ascites were observed. The liver was congested and mottled yellow white intermingled with randomly distributed pin-point to patches of red foci. Brush hemorrhage was noted in the sub-aortic endocardium, bicuspid valve, and papillary muscle of the left ventricle of the heart. The lungs were diffusely mottled red with prominent emphysema along the edge of most lobes. The mucosa of the proximal duodenum had locally extensive ecchymosis and petechiation and the affected segment was filled with a moderate amount of red, mucoid content.

**Histopathological Findings:**

**Liver:** There is diffuse, severe centriflobular hemorrhage and necrosis along with moderate peribular fatty change in all lobules. Small numbers of neutrophils along with variable amounts of brown pigment and cell debris are present in the centrlobular region. Hypertrophied Kupffer cells are also noted.

**Spleen:** There are mild lymphoid depletion, multifocal hemorrhage, and brown pigment deposition along with variable numbers of megakaryocytes and mild RE hyperplasia in the red pulp. The special stain, Berline blue, confirms the presence of large amounts of iron pigment.

**Kidney:** Multifocal, moderate, hydropic degeneration and coagulative necrosis are seen in the tubular epithelial cells along with the presence of hyaline casts in the lumen.

**Heart:** There are areas of mild to moderate fibrosis with myofiber atrophy and interstitial edema in the myocardium combined with areas of mild endocardial hemorrhage. Trichrome staining confirms areas of myocardial fibrosis. Infrequently, there are scattered lymphocytes and plasma cells infiltrating the interstitium.

**Lungs:** There are moderate atelectasis and emphysema of the alveolar spaces accompanied with scant mononuclear inflammatory cell infiltration.

**Histochemical Stain:**

Berline blue confirms the presence of large amounts of iron pigment in the spleen while no positive granules were seen in the liver. It is considered that due to the acidic environment in the liver, ferric (Fe³⁺) iron deposited in liver is converted to ferrous (Fe²⁺) iron which disables the binding with ferrocyanide (berline blue) resulting in the negative result in berlin blue stained-liver.

**Discussion:** The histopathology of iron overload in the lemur has been well described. Clusters of hemosiderin occur throughout the mononuclear-phagocytic cell system in the duodenum, liver, and spleen. This disease progresses in older animals to a generalized hemosiderosis in which the iron also appears in the phagocytic cells of the kidney, bone marrow, and lungs; in severe cases, hemosiderin
also accumulates in the parenchymal cells and interstitial areas of various organs, particularly in the liver.

At the San Diego Zoo, excessive tissue iron deposits were found in all mature lemurs necropsied between 1968 and 1987, including the ring-tailed lemur, black lemur, brown lemur, collared lemur, black and white ruffed lemur, and sifaka. In that report, younger lemurs had trace amounts of hemosiderin in the duodenum, liver, and spleen while the older animals had extensive iron overload throughout the gastrointestinal tract and in a variety of other tissues. In older animals, the hemosiderosis appeared heaviest in the duodenum but was also recognizable in the stomach, jejunum, and ileum. This is the most well-known publication by Spelman et al. (1989). They also reported that hemosiderosis in the lemur was closely associated with liver and kidney diseases. The liver was characterized most often by fibrosis, hepatocellular necrosis, distorted architecture, bile duct hyperplasia, hepatocellular fatty degeneration, and congestion. The kidney had renal necrosis and/or glomerulonephritis. The iron accumulation was considered as a serious and persistent health threat to captive lemurs.

Iron absorption and transport into the systemic circulation occurs only in the duodenum and upper jejunum. Iron from diets can be classified into three absorbable forms: heme, ferric (Fe³⁺) and ferrous (Fe²⁺). Heme is the most efficient form for absorption. As regards to ferric (Fe³⁺) and ferrous (Fe²⁺) iron, they need special proteins to transport into or between cells and absorption efficiency changes easily due to diets. Iron is transported to cells via blood by binding with transferrin for body use or stored as a stable substance, ferritin, in cells. The non-pathologic accumulation of iron in tissues is called hemosiderosis. However, the pathologic accumulation of iron in tissues with functional or morphologic evidences of iron intoxication is called hemochromatosis.

Iron intoxication in leaf-eating monkeys has been 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. Lemur is a unique species and has an unusual preference for diets containing high tannin that prevents the excess absorption of iron. But compared with wild lemurs, the diets of captive lemurs are high in iron, high in ascorbic acid, but low in tannin. Tannins are polyphenols that inhibit iron absorption by acting as natural chelators in the GI tract; dietary iron is bound to hydroxyl groups and thus prevented from uptake by mucosal cells. Iron toxicity results from free-radical formation, which increases damages to cell membranes by lipid peroxidation and protein cross-linking. High ascorbic acid enhances tissue damage by free-radical formation. The captive diet may trigger the occurrence of iron intoxication.

Thus, diets readjustment along with periodic complete physical examinations and blood iron analysis could effectively avoid excess-iron absorption and establish normal blood iron reference values for health monitoring in captive lemurs.

Three cases of iron intoxication in ring-tailed lemur happened suddenly in Taipei Zoo in 2005. The clinical signs included lethargy and sudden death with the accumulation of a large amount of iron and necroses in various organs via pathological examination. The recommendation of dietary modification prevented further incidence of the disease.

References

Bacterial Septicemia in a Mongolian Wild Horse (Equus przewalskii)

K. C. Chui¹, S.-H. Hsiao¹,², V.-F. Pang¹,², C.-H. Liu¹,², F.-I. Wang¹,², C.-R. Jeng¹,²

¹Graduate Institute of Veterinary Medicine, National Taiwan University
²School of Veterinary Medicine, National Taiwan University *Corresponding author: shsiao1@ntu.edu.tw

Keywords: horse, Mongolain, septicemia

Clinical History: A one-day old male Mongolian wild horse (Equus przewalskii), was submitted from Taipei City Zoo. The animal was born at 8:30 A.M. on February 10, 2009. After delivery, the foal showed good spirit and was kept in closely observing until 18:20 P.M. On next morning, 7:15 A.M., the animal was on lateral recumbency and couldn't stand up after help. An emergency treatment was performed but the animal was not response.

Diagnosis: Suspected septicemia in a Mongolian wild horse (Equus przewalskii)

Gross Findings: Multifocal to coalescing, 1-3mm pale foci in renal cortex of both kidneys were noted. There was diffuse hyperemia to hemorrhagic lesion at cortex of right adrenal gland. There was significant demarcation between gastric fundus and cardiac region, the mucosa surface of fundus appeared diffusely dark reddish. Ingesta was clear and contained with forage. The mucosa from entire duodenum to colon appeared diffusely dark reddish to hemorrhagic. Subcutaneous tissues were muddy reddish yellow and speckled with petechial and ecchymotic hemorrhages.

Histopathological Findings: Multifocal accumulations of basophilic bacterial colonies in the renal glomeruli associated with congestion are noted in the kidneys. The adrenal cortex is diffusely, severely congestion and hemorrhage. The basophilic bacterial colonies are noticeable in the lesions. Fibrinous clots are noted in the blood vessels of the lung, kidney and omentum. The amounts of cells in both thymus and spleen become lesser, accompanied with hyperemia or congestion. There is congestion in the stomach and intestines.

Laboratory Results

Brown and Brenn Gram staining method: Clumps of Gram negative coccobacilli were detected in the kidney by the Brown and Brenn Gram staining method.

Polymerase chain reaction: PCR analysis is now on proceeding.

Discussion: Septicemia is an important cause of morbidity and mortality in neonatal foals and is characterized as an etiology. A variety of factors predispose foals to septicemia, nevertheless, failure of passive transfer of immunoglobulins being the most common. Weak foals and foals that fail to obtain colostrums being at risk. The possibility of immunodeficiency like Arabian foal should be considered. There are minor regional differences in disease prevalency with which various bacterial species are isolated from septicemic foals, and the role of gram-negative bacteria appears to have increased in recent years.

Early studies by Dimock et al on American studfarms and by Miller on British studfarms indicated that Actinobacillus equuli and Streptococcus spp were by far the predominant isolates from infected foals. Later studies in the Newmarket area of England, first by Platt in 1973 and later by Whitwell in 1980, indicated that E.coli was important etiologic agent of foal septicemia and that it was isolated with greater frequency than was A. equuli, the second most frequent isolate. These authors also reported that streptococcus spp and other gram-positive bacteria were infrequently isolated in pure culture from septicemic foals.

In this case, the differential diagnosis for bacterial septicemia in the foal include Actinobacillus, Escherichia coli, Streptococcus, Salmonella, Rhodococcus equi etc.

Within the general background, specific changes may suggest an etiological diagnosis. Focal accumulations of basophilic bacteria in the renal glomeruli and in the capillaries of the adrenals and often, other organs, is characteristic of Actinobacillus equuli septicemia. The synovitis with this infection is serofibrinous and tined with blood, rather than frankly purulent as with Streptococcus zooepidemicus and Salmonella spp. E. coli septicemia is marked by severe, hemorrhagic inflammation of, particularly, the large intestine. This is uncommon and characteristically seen in the very young foal, often in association with severe hemorrhagic metritis of the mare. Rhodococcus equi infection is generally confined to the lungs and/or large intestine, without evidence of septicemia. Salmonellosis is and epizootic disease in foals. Severe cattarhal colitis is seen with erosion and ulceration of the mucosa in prolonged cases.

The age of the affected foal is a guide to probable etiology. A. equuli and E. coli septicemias invariably occur within a week or so of birth. Streptococcal septicemias can appear this early, but are more common from about 3 weeks to 3-4 months of age. R. equi is not often seen before 2-3 months. Salmonellosis may occur anytime from about a month to 3-4 months. Its epizootic nature is characteristic.

References

Cryptococcosis in a Cat

T. Yanai1*, M. Murakami1, Y. Tatikawa1, H. Sakai1, R. Kano2

1Department of Veterinary Pathology, Gifu University  2Department of Clinical Pathology, Nihon University  
*Corresponding author: yanai@gifu-u.ac.jp

Keywords: cat, cryptococcosis, granuloma

Clinical History:
A 2-year-old female mixed-breed spayed cat had a nodular lesion involving the subcutis in the left mandibular region. The owner noticed the mandibular nodule one month before submission, which showed rapid growth, and then ruptured. At first the clinician suspected lymphoma originating from the mandibular lymph nodes, and the nodule was removed surgically for histopathological diagnosis.

Diagnosis:
Cryptococcosis

Gross Findings:
The resected subcutaneous nodule was round in shape, approximately 8 cm in diameter, partially covered by the skin and surrounding connective tissue. The cut surface of the nodule was grayish-white in color and soft in texture, and was well-demarcated from surrounding tissue. The nodule was expanded deeply and involving muscle and fatty tissues, as well as lymph nodes.

Histopathological Findings:
Microscopically, subcutaneous nodule consisted of a diffuse infiltration of a large amount of macrophages, forming dispersed granulomatous lesion with various degrees of necrosis in the subcutaneous tissue and muscle layer. There were numerous yeast-like cells which were surrounded by wide unstained clear spaces like “halos” in the granulomatous lesions and necrotic areas. They were usually seen within epithelioid histiocytes and multinucleated giant cells. The yeast-like cells were 4 to 10 micro meter in diameter, and spherical to oval in shape and uninucleated. The fungal cells had occasional buds. Under PAS reaction, the yeast-like cells were strongly positive. On the surface of the skin with the nodule, fungal cells were shedding from erosion of the skin where the granulomatous lesion was opened directly to the outside.

Discussion
Based on morphological features of spherical to oval, various-sized yeast-like cells with surrounding halo-like space, the present case was diagnosed as cutaneous cryptococcosis caused by Cryptococcus neoforman, a soil-inhabiting yeast-like fungus that is abundant in avian habitat. Although cryptococcosis is the most common systemic fungal infection in cats with subcutaneous nodules, respiratory tract disease, lymphadenopathy, intraocular inflammation, or CNS disease (1, 2) the present case is rather unique because of a larger subcutaneous nodule which had some difficulty to distinguish from lymphomas clinically.

References
**Mycobacteriosis in a Miniature Schnauzer Dog: A Case Report**

S. Theerawatanasirikul, W. Banlunara*, P. Teewasutrakul, S. Puranaveja

*Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand  *Corresponding author: wijit.k@chula.ac.th

**Keywords:** dog, lymphoma, mycobacteriosis

**Clinical History:** A 6-year-old spayed female, miniature Schnauzer dog was presented at the Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University with the signs of mild anorexia, depression and generalized lymph nodes enlargement. Clinical examination found fever, hepatosplenomegaly and superficial lymph nodes enlargement. Multifocal masses at upper and lower lips, the right elbow and the cranial part of the right hind limb were also developed after 3 months of the treatment.

**Diagnosis:** Canine cutaneous lymphoma
Generalized mycobacteriosis

**Gross findings:** Generalized lymph node enlargement with multifocal round to oval subcutaneous masses were varied about 0.3-1.2 cm. in diameter. The cut surface of the biopsy skin masses were pale white-pinkish, soft to firm consistency.

**Histopathological findings**

**Lymph node:** Cytology and histopathology revealed extensive infiltration of active histiocytes and multinucleated giant cells, which engulfed many translucent rod shaped bacteria in their cytoplasm. Moderate infiltration of neutrophils and ruptured lymphoid follicles with severe depletion of the lymphocytes.

**Skin:** The skin biopsy at cranial part of the right hind limb showed massive infiltration of large foamy macrophages and multinucleated giant cells, which contained numerous clear, rods, shiny bacilli bacteria in the cytoplasm, in the dermis. Few numbers of lymphocytes and plasma cells infiltrated along the fibrous tissue.

**Special histochemical staining:**

The Ziehl-Neelsen acid-fast staining of the cytology and histopathological sections demonstrated many acid-fast-positive bacilli in the cytoplasm of histiocytes and multinucleated giant cells. In cytology, there were many free acid fast-positive bacilli on the background. The bacilli were 3-4 μm-long.

**Bacterial culture, PCR and DNA sequencing**

The fastidious bacterial cultures, polymerase chain reaction (PCR) and DNA sequencing were taken. The bacterial culture result was non-photochromogen of Mycobacterium species complex with the drug sensitivity to Rifampin. The PCR and DNA sequencing was performed with using 16sRNA mycobacterial primers and DNA sequences was analysed and the result was relatively to *Mycobacterium avium*.

**Discussion**

Although zoonotic mycobacterial infections are uncommon in dogs, there still have been reported more susceptible to infection in dogs, such as *M. tuberculosis* and *M. bovis* infection. Most dogs apparently resistant to mycobacteria, especially *Mycobacterium avium* complex (MAC). The previous reports showed miniature schnauzer dogs were susceptible to *M. avium* infection. Age of onset of known cases seems to be from 10 months to 3 years. The primary symptoms of disseminated *Mycobacterium avium* infection are enlarged lymph nodes, and inappetence or anorexia. Other symptoms may also include fever, vomiting, enlargement of spleen and liver, and lameness. There may have been misdiagnosed as canine lymphoma or malignant histiocyma. It is possible that certain dog breeds could have genetic mutation that predispose to *M. avium* infection or inherited immune system dysfunction for killing of intracellular bacteria and could be straightforwardly prone to MAC infection. To achieve definitive diagnosis, bacterial culture on fresh tissue samples followed by the use of molecular genetics techniques or PCR on formalin-fixed tissue are required. The DNA sequencing and phenotypic characteristics were also suggested that this strain is representatives of mycobacterium species.

**References**

Mucinous Gastric Adenocarcinoma in a Dog

N. Charoenvisal*, W. Banlunara

Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, 10330 Thailand

*Corresponding author: earn_na_taya@hotmail.com

Keywords: adenocarcinoma, dog, stomach

Clinical history: A 7-years-old, female, golden retriever dog had continuously weight loss (decreasing of 5 kg within 6 weeks). The dog showed the clinical signs of anorexia and depress. Veterinarian palpated the abdomen and suspected that there is a mass at right cranial abdomen. Radiographic examination showed heterogeneous fat density at cranioventral abdomen and unclear caudal stomach lining. Ultrasonography revealed the thickening of stomach and duodenal wall. The surgical excision of the mass was performed. Unfortunately, the dog was died after the operation finished.

Diagnosis: Mucinous adenocarcinoma

Gross findings: The stomach showed mucosal edema and hemorrhage with a large mass at pyloric area. Marked mesenteric lymph node enlargement and diffuse hemorrhagic catarrhal enteritis of duodenum and jejunum with edema were found. Liver had greenish-gray in color. Kidney showed white streaks at corticomedullary junction.

Histological findings: At lower magnification, the lesions showed numerous of large irregular glandular structures infiltrated in gastric mucosa, muscularis mucosae and surrounding adipose tissue. The glandular structures consisted of acini with over production of mucin, which seen as basophilic intracytoplasmic vacuoles that was positive with alcian blue staining. The excessive mucin production caused cells rupture and form lake of mucin.

Discussion: Adenocarcinoma of gastrointestinal tract is not common in dog compared to the incident in human. However, it can be occur at pyloric part of the stomach, colon and rectum and sometime in duodenum, jejunum. The average age of affected dogs is 7.5-10.2 years old. Gastric adenocarcinoma can be classified by histological features into 4 types; tubular adenocarcinoma, mucinous adenocarcinoma, signet ring cell carcinoma and solid or undifferentiated carcinoma. Tubular adenocarcinoma composed of tubular structure with or without papillary projection. Mucinous adenocarcinoma showed marked mucin production as basophilic intracytoplasmic granules. The third is signet ring cell carcinoma consisted of eccentric nuclei tumor cells with distended cytoplasm filled with mucin. The gastric adenocarcinomas that have no glandular structure are classified as undifferentiated or solid carcinoma.

Dogs that suffered from gastric adenocarcinoma show only non-specific clinical signs, such as weight loss, anorexia, diarrhea, melena and dullness. Therefore, clinical presentation is often late with large tumor or extensive involvement of gastric mucosa or deeper layer. The metastasis from primary gastric carcinoma should be expected because the diagnosis is made relatively late in the progression of tumor. Treatment with surgical remove is the best choice but recurrence neoplasm can be found. Radiation and chemotherapy were not much help due to the stage of disease. Prognosis is poor and average survival time is 2 months.

In the present case, the dog present at the hospital without specific clinical signs but the large mass at pyloric area were seen and removed during the operation. Later, it had been diagnosed as a gastric mucinous adenocarcinoma with metastasis tumor to pancreas, omentum and lymph node. However, in our laboratory this tumor is very rare, whereas, the gastric leiomyoma is commonly found but it causes less complication and no metastasis.

References

Adenoid Basal Cell Carcinoma in a Horse

N.Y. Park1, M.I Kang1, H.M. Na3, Y.T. Cho1, J.W. Choi1, S.H. Lee1, J.H. Lee2, C. Choi2

1Department of Veterinary Pathology, College of Veterinary Medicine, Chonnam National University, Gwangju, 500-757, Republic of Korea 2Department of Pathology, Medical School, Hwasun Hospital, Chonnam National University, Hwasun, Republic of Korea 3Gwangju Health and Environment Research Institute, Gwangju, Republic of Korea

*Corresponding author: nypark@hanmail.net

Keywords: Basal cell carcinoma, horse, immunohistochemistry

Clinical History: A 12-year-old male Thoroughbred horse presented and showed photophobia and purulent ocular discharge from the right medial canthus. This horse was referred to our school for necropsy lesson for undergraduate students.

Diagnosis:
Adenoid Basal Cell Carcinoma

Gross Findings: The horse was euthanized and a mass was found in the third eyelid. The size of the mass was 0.7x1.5x0.3 mm and had pinkish red color. There was purulent inflammatory exudates severely discharged from the right eye.

Histopathological Findings: The tumor was well demarcated and the superficial area was ulcerated under a microscope with an H&E stain. Morphologically, it was slightly palisading shape in the periphery and adenoid structure (glandular differentiation) in the center. Based on this morphological feature, we diagnosed the horse as having adenoid basal cell carcinoma.

Immunohistochemical Results: Based on this morphological feature, we had to rule out adenoid basal cell carcinoma, sebaceous carcinoma and eccrine/apocrine carcinoma. In order to make specific diagnosis, an immune stain was performed. The tumor markers used were CEA, EMA, CD15, and Ki-67. Results were negative for CEA, EMA and CD 15 stains and weak positive for the Ki-67 stain, however, it did not reach statistical significance (100%). In conclusion, we diagnosed the horse as having adenoid basal cell carcinoma based on both the negative response on EMA and CEA stains and by the morphology, respectively.

Discussion: Basal cell carcinoma is rare type of cancer that affects the eye of the horse. This type of cancer would be common form amongst humans but the adenoid type is not so common in humans yet. Basal cell carcinoma in horses is well defined in the disease of horse text but Adenoid type of this cancer is not described yet.

This case was meaningful to report because Adenoid Basal Cell Carcinoma is very rare in horses and also it rarely occurs in the third eyelid.

References
Inflammatory Myofibroblastic Tumor in a Amazon Jaguar

N.Y. Park¹*, M.I. Kang¹, J.W. Choi¹, Y.G. Yeo², Y.M. Jeong², E.W. Mo², J.H. Lee³, C. Choi³

¹Department of Veterinary Pathology, College of Veterinary Medicine, Chonnam National University, Gwangju, 500-757, Republic of Korea  ²Seoul Zoo, Seoul Grand Park, Gwacheon, Gyeonggi-do, Republic of Korea  ³Department of Pathology, Medical School, Hwansun-hospital, Chonnam National University, Hwasun, Republic of Korea  *Corresponding author: nypark@hanmail.net

Keywords: amazon jaguar, myofibroblast, tumor

Clinical history: A 10-years-old, female amazon jaguar (Panthera onca) in Seoul Zoo, which had a mass around the elbow joint on left forelimb. There was big ulceration on the center of tumor mass due to continuous licking on the lesion. Surgery was carried out to remove the tumor mass. She lived for a while after the surgery but died recently.

Diagnosis: Inflammatory myofibroblastic tumor

Gross Findings: Overall size of the tumor mass was 11 cm (length) x 7 cm (width) as observed. There was 5 cm (length) x 5 cm (width) round ulceration located on the middle of the tumor mass.

Histopathological Findings: It was consisted of interweaving streams of bland spindle cells among which numerous lymphocytes were scattered. All tumor cells exhibits myofibroblastic (myoid) features but that cells is not plumped spindle cells. There was no storiform appearance in the finding. We needed to rule out leiomyosarcoma, rhabdomyosarcoma and fibro-histiocytic tumors.

Immunohistochemical Results: We used four different markers for immunohistochemistry stain: actin, desmin, calponin and caldesmon. Immunohistochemistry results were consistent with a myofibroblastic derivation for the spindle cell population and the diagnosis of inflammatory myofibroblastic tumor was made. In the finding, the tumor cell expressed the following reaction: strong positive for desmin, showed obscure reaction by actin, strong positive with calponin and negative with caldesmin.

Discussion: Inflammatory myofibroblastic tumor(IMT) are discrete masses composed of a mixture of bland fusiform myofibroblastic cells and an inflammatory infiltrate composed of varying proportions of lymphocytes and etc. They are well described in humans and occur most commonly in the lungs of children and young adults.

Myofibroblastic tumor is difficult to classify in the tumor class. Certain pathologists have taken restrictive view toward definition of myofibroblast, while others use the term to explain any cells having features of both fibroblast and smooth muscle cells.

According to the immunohistochemistry point of view, it is well-known that myofibrosarcoma express calponin but not caldesmon, whereas leiomyosarcoma are said to exhibit both calponin and caldesmon. We could not find any detailed information on inflammatory myofibroblastic tumor in the soft tissue from any veterinary pathology books and WHO Histological classification of Domestic Animals.

Therefore, this case was concluded as inflammatory myofibroblastic tumor of Amazon jaguar.

References