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INSTRUCTIONS TO AUTHORS

The Thai Journal of Veterinary Medicine publishes articles reporting interdisciplinary investigations concerning veterinary and animal sciences, at all levels of resolution, from basic to clinical, molecular to behavioral, and opinions that are of general interest to the broad community of veterinarians and biological scientists. Clinical or pathological investigations, protocols and reviews will also be considered for publication if they provide significant insight into the structure or function, the pathophysiology of a disease, or its treatment.

In the Journal’s Table of Contents, published articles will be shown under one of the appropriate Section titles listed below.

SECTIONS

Editorials: A limited amount of space will be available for comments about important scientific points or subjects of topical interest, and will be by invitation only.

Reviews and Minireviews will be either by invitation, or submission. The latter will be reviewed by experts in the same manner as other submitted manuscripts.

Original Articles should be novel research findings and provide strong evidence for the conclusions. The manuscripts suitable for publication in TJVM should be of extreme importance to scientists in the field as well as interesting to researchers in other disciplines.

Rapid or Short Communications: These are short communications that describe outstanding new discoveries. This decision will be based on whether the paper reports particularly important findings that are likely to have a high impact in the field of work.

Clinical or Pathological Reports: These are short reports of original clinical or pathological findings whose importance mean that they will be of interest to veterinarians.

Diagnostic Forum is a regular feature of TJVM. This includes Ultrasound Diagnosis, ECG Quiz, Ophthalmology Snapshot and What Is Your Diagnosis, all of which will be by invitation only.

SUBMISSION POLICY

Submission of a paper to TJVM is understood to imply that it deals with original material not previously published, and that it is not being considered for publication elsewhere. Please write your text in good English (American or British usage is accepted, but not a mixture of these). The layout and style should adhere strictly to the instructions given under “Organisation of the Article”. Four copies of the manuscripts should be submitted to the Editorial Board, The Thai Journal of Veterinary Medicine, Chulalongkorn University, Bangkok 10330, Thailand. Fax: 02 218 9677. The final version of the manuscript including all figures and tables should be submitted in both hard copy and digital form. No revisions or updates will be incorporated after the article has been accepted and sent to the Publisher (unless approved by the Editorial Board). The Editorial Board reserves the right to reject any manuscript deemed unsuitable for publication in TJVM.

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SUBMISSION PROCEDURE

Authors are requested to submit their manuscripts in English, with abstracts in both Thai and English, in a concise and understandable style. Technical jargon or “laboratory slang” should not be used. Please note that the electronic files supplied will always be used to produce the illustrations, including those for the print version of the article; it is the Authors’ responsibility to ensure that the manuscript is written in a style that is grammatically correct and free of spelling or other typographical errors, and that these files are of suitable quality.

All manuscripts must be typewritten using TIMES font at 12 point, with double-spacing throughout and with margins at least 2.5 cm wide. Pages should be numbered in succession, the title page being no. 1. Text files should be supplied in Windows Microsoft Word or Word Perfect formats.
Each manuscript should be accompanied by a signed cover letter in which the corresponding Author states: “The work described has not been submitted elsewhere for publication, in whole or in part”. All submissions to TJVM must contain experiments that conform to the ethical standards issued by the National Research Council. If the studies deal with animal experiments, the authors certify in the Materials and Methods section, that the procedures have been approved by the Authors’ Institution's Ethic Committee, and care was taken to minimize the number of animals used. If the ethical standard governing the reported research is different from those guidelines indicated above, the authors must provide information in the submission cover letter about which guidelines and oversight procedures were followed.

Authors should only use abbreviations sparingly and should always define the abbreviation when first used in the text by placing it in parentheses after the full term. The abbreviations should then be used consistently thereafter and appear at least twice in the text. Drug names should be the official or approved names; trade names or common names may be given where the drug is first mentioned. Trade names should be capitalised and the manufacturer’s name and country given in parenthesis thereafter. The doses of the drugs should be given as unit weight/unit body weight, e.g. mmol/kg or mg/kg. Symbols for physical units should be restricted to the Systems Internationale (S.I.) Units.

Where possible, Authors should also include a list of three or more potential reviewers for their manuscript, with contact information.

**ORGANISATION OF THE ARTICLE**

The manuscript should have a uniform style and be submitted exactly as the author wishes it to appear in print. It should consist of subdivisions in the following sequence.

1) Title page
2) Abstract
3) Text
4) Acknowledgments
5) References
6) Tables
7) Figures legends
8) Figures

Start each subdivision on a new page.

**Title Page.** The first page of the manuscript should include:

- Title of paper
- Full name of author(s)
- Institutional affiliations and complete mailing address
- The exact number of pages, figures and tables in the article
- Individual, address, and telephone number to whom correspondence concerning manuscript should be sent.

**Abstract.** Submit an abstract of around 250 words that will serve in lieu of a concluding summary. The abstract must be written in complete sentences and succinctly state the objectives, experimental design of the paper, principal observations, and conclusions; it should be intelligible without reference to the rest of the paper.

**Key Words.** Four to six key words should be included.

**Text.**

- **Original articles:** The text should be presented in the following order: INTRODUCTION; MATERIALS AND METHODS; RESULTS; DISCUSSION.
  
  (i) **Introduction.** This should provide the scientific rationale for the research that is reported. No results should be presented.

  (ii) **Materials and Methods.** Procedures used in the research should be described in sufficient detail to permit the replication of the work by others. Previously published procedures should be referenced and briefly summarised. The source of all materials, including animals and human tissue, must be provided.

  (iii) **Results.** This section presents findings without discussion of their significance. Subsections should be used in order to present results in an organised fashion. The findings may be assisted by high quality illustrations, as necessary, to adequately document the work. Figures should be referred to in the text as Fig.1, Figs. 1, 3-4, etc., and tables as Table 1, Table 1, 3-4, etc.

  (iv) **Discussion.** This section presents the Authors’ interpretations of their findings and an assessment of their significance in relation to previous work. Repetition of material presented in the Results section should be avoided.

- **Short Communication and Clinical or Pathological Reports:** These should not exceed 4 pages (approximately 2,000 words in total, including spaces) Follow the instructions for Original Articles with the
exception that results and discussion are combined.

- **Reviews**: Reviews should have an introductory section, followed by several information presentation sections and end with a conclusion section. Section headings should be used to organise the presentation of information.

**Acknowledgements.** It is the corresponding Author’s responsibility to ensure that individuals who are acknowledged for assistance or for providing comments on the manuscript are agreeable to being acknowledged in this way.

**References**

(a) In the text, references should be quoted as the name of the first author and year in chronological order. Multiple authors are indicated by “et al.”, except when there are only two authors, in which case both names are written.

*Examples:*

..... (Garthwaite and Garthwaite, 1995; Morris, 2000).

..... by Nagy et al. (1999a,b).

Clarkson et al. (2004) stated......

(b) The reference list should be on a separate page at the end of the manuscript, in alphabetical order and arranged as follows: authors’ names and initials, year, title of the article, abbreviated title of the journal, volume, first and last page numbers.

*Examples:*

**Article in a periodical:**


**Chapter in a book:**


**An entire book:**


**Electronic information:**


(c) A paper which has been accepted for publication but has not yet appeared may be cited in the reference list with the abbreviated name of the journal followed by the words “in press”. Avoid using abstracts, theses or dissertations as references. Unpublished observations and personal communication may not be used as references.

(d) Unpublished or submitted experiments by one of the authors may be mentioned only in the text, not in the References. Initials, as well as surnames, must be given for authors whose unpublished experiments are quoted: (M.L. King, unpublished observations)

**Tables or figures.** These should be included on separate pages placed at the end of the manuscript. Their desired approximate locations should be indicated in the text. Each figure must be accompanied by an explanatory legend in a separate section entitled Figure Legends. In general, tables and figures should be constructed so that they, together with their captions and legends, will be intelligible with minimal reference to the main text. Table and figure legends should be written as in the following examples.

**Figure 1** Typical lesions of...

**Table 1** Statistical analysis showing....

All graphic files must be submitted, as JPEG, in sufficiently high resolution (300 dpi for grayscale or colour images and 600-1000 dpi for line art) to allow for printing. Keep text and graphics (and any other items) as separate files - do not import the figures into the text file. Name your files using the correct extension, e.g. text.doc, fig1a.eps, fig1.tif, Fig1.jpg, tbl1-6.xls, etc. Authors will be required to pay a fee towards the extra costs incurred in colour printing.

Authors will be required to pay a submission fee, 1500 baht for original articles or review articles, 1000 baht for short communications.
The Asian Society of Veterinary Pathologists (ASVP)

Achariya Sailasuta

ASVP’s vision will be recognised by the public as an unified Veterinary Pathology of the region and the mission is to enhance the academic improvement in the region through responsible of Veterinary Pathologists.

The Asian Society of Veterinary Pathologists (ASVP) is a society of the Asian Veterinary Pathologists. The activities are regularly promoting academic activities and collaborating the veterinary service for the improvement of veterinary pathology in the region. The ASVP was firstly founded in Japan by The Japanese Society of Veterinary Pathologists (JSVP) and the 1st ASVP meeting was held by Prof. Okada in Tokyo, Japan in 2003. The meeting was held every two years as the 2nd was in Seoul, Korea in 2005 hosted by KSVP. And the 3rd ASVP was held in Taipei, Taiwan in 2007 which hosted by TSVP.

The ASVP’s members are Japanese Society of Veterinary Pathologists (JSVP), Indonesian Society of Veterinary Pathologists (ISVP), Veterinary Pathologists Society of Malaysia (VPSM), Korean Society of Veterinary Pathologists (KSVP), Philippines Society of Veterinary Pathologists (PSVP), Taiwan Society of Veterinary Pathologists (TSVP), Thai Society of Veterinary Pathologists (TSVP) and Vietnamese Society of Veterinary Pathologists (VSVP). The present president of ASVP is Assoc. Prof. Dr. Achariya Sailasuta. The president of Thai Society of Veterinary Pathologists and is prouded to host the 4th ASVP in this coming 19-20 November 2009, at the Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand.

The ASVP’S values are to coordinate and facilitate academic assistance for the member societies, to establish mutually beneficial relationships with local and international sectors, to encourage veterinary pathologists to provide quality veterinary pathology service and educate the public i.e ASVP meeting every two years, extending the academic information and arranging the training program that facilitate improvement of specialized veterinary pathology education relevant to the region.

ASVP has now developed a linkage with the Federation of the Asian Veterinary Associations (FAVA) and the Asian Zoo and Wildlife Medicine and Conservation (AZWMC). These connection need to continue to exploit their influence through linkages, sharing of information and ideas. In this way, ASVP can work together to achieve our visions of being a unified professional veterinary pathologists servicing the veterinary needs of the region. Finally, on behalf of ASVP and TSVP, we wish to thank all member societies for their valuable contributions. We realize that it is sometimes difficult to contribute, but everyone has always done their best and we can be proud of our collective achievements. By the year 2009, It is a great opportunity for the Thai Society of Veterinary Pathologists (TSVP) to host the 4th Asian Society of Veterinary Pathologist Conference (4th ASVP). The conference is in cooperation with the Department of Veterinary Pathology, Faculty of Veterinary Science, Chulalongkorn University, the Thai Association of the Veterinary Laboratory
Diagnosticians (TAVLD), the Zoo Organization under the Royal Patronage, the Animal Oncology Center, the Innovation Center for Veterinary Teaching and Services in the Companion Animal, Asian Foundation for the Advancement of Veterinary and Animal Science (AFAVAS), CL Davis Foundation for the Advancement of the Veterinary Pathology, The Federation of Asian Veterinary Associations (FAVA), Asian Society Wildlife Pathology and Parasitology (ASWPP) and the National Taiwan University, is scheduled to be held on 19-20 November 2009 at Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand, in the theme of *Era of the Modern Diagnostic Pathology*. The objectives of the conference are; to promote the co-operation of regional and global Agro veterinary-industry sectors, to provide an academic exchange among the veterinary pathologists and to build up the connection for the diseases monitoring; zoonosis and emerging diseases in the country and the region. Please Further informations kindly visit, http://www.vet.chula.ac.th/path
In Vitro Efficacy of Human-Derived Probiotic, \textit{Lactobacillus rhamnosus} Against Pathogenic Bacteria in Fish and Frogs

Nopadon Pirarat$^1$ Komkiew Pinpimai$^1$ Katriya Chankow$^1$ Kotchakorn Malila$^1$
Nantrika Chansue$^2$ Waree Niyomtham$^3$ Channarong Rodkhum$^3^*$

Abstract

Probiotic supplementation is now being focused as an alternative method to control fish diseases worldwide. This study investigates the \textit{in vitro} efficacy of a human-derived probiotic, \textit{Lactobacillus rhamnosus}. The first results of the screening for antimicrobial activity using agar spot test and disc diffusion showed that \textit{L. rhamnosus} has a broad range against twelve isolates (n=12) of Gram-positive and Gram-negative pathogenic bacteria in fish and frogs: \textit{Streptococcus iniae} (n=4), \textit{Streptococcus agalactiae} (n=3), \textit{Aeromonas hydrophila} (n=3), \textit{Chryseobacterium indologenes} (n=1) and \textit{Edwardsiella tarda} (n=1). Agar spot test on killed probiotic bacteria indicated that only the metabolic product of probiotic is involved in the growth inhibition of pathogenic bacteria. When confirmed by a co-culture study, the growth of all pathogenic bacteria that were cultured with a probiotic was lower than the control. All the findings suggest that \textit{L. rhamnosus} has a high potential for inhibiting the growth of pathogenic bacteria in fish and frogs.

\textbf{Keywords} : fish, frog, human-derived probiotic, \textit{Lactobacillus rhamnosus}, pathogenic bacteria

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Introduction

Among the causes of economic loss in aquaculture, infectious disease from bacteria is one of the most important. The key to success in preventing and controlling infectious diseases depends on several factors: the host immunity, the pathogen and also the environment. Two common methods of preventing and controlling are vaccination and chemotherapy, each of which can deal with only one factor. Accordingly, probiotics have become an interesting alternative way because of their direct ability to inhibit the growth of other microorganisms and modulate the host’s immunity (Fuller, 1989; Gatesoupe, 1999; Verschuere et al., 2000). In the aquatic system, where hosts and pathogens share the same ecosystem, hosts are fully exposed to the pathogens (Verschuere et al., 2000). The direct effect such as inhibiting the growth of other organisms might be the main action that can occur in cultured system (Kesarcodi-Watson et al., 2008) and the ability to produce inhibitory compounds of live probiotic bacteria is one of the important actions that results in the growth inhibition of other microorganisms (Balcazar et al., 2006; Kesarcodi-Watson et al., 2008). Among probiotic candidates, Lactobacilli have the longest history of use (Fuller, 1989). Various health effects for human have been attributed to Lactobacillus rhamnosus, such as the prevention of acute diarrhoea in children, the prevention of antibiotic-associated diarrhoea and the prevention and treatment of allergies, lowering cholesterol levels and the immune stimulation (Majamaa and Isolauri, 1997; Anuradha et al., 2005). In recent years, there has been great interest in the use of lactic acid bacteria (LAB) and their metabolic products as potential probiotics in aquaculture since LAB probiotics are considered safe for fish as a human food and have the ability to fight against harmful pathogens directly and indirectly (Gatesoupe, 2008). The objective of the present
study is to determine the in vitro efficacy of *Lactobacillus rhamnosus*, a human-derived probiotic that has been used in humans to control gastrointestinal diseases and some bacterial-infectious diseases of fish and frogs.

**Materials and Methods**

**Bacterial strain and culture medium**

*L. rhamnosus* (ATCC 53103) was cultured at 37°C on De Man, Rogosa and Sharpe (MRS) agar with 0.3% CaCO₃ and modified-MRS medium (M-MRS medium), a suitable medium for lactic acid bacteria. Twelve isolates of pathogenic bacteria (n=12) were cultured at 30°C on trypticase soy agar (TSA) and on trypticase soy broth (TSB). All pathogenic bacteria were isolated from organs of diseased fishes and frogs in Thailand and Japan (Table 1). All isolated bacteria were identified by the conventional biochemical method and confirmed by polymerase chain reaction (PCR).

**Agar spot test with live probiotic bacteria**

*L. rhamnosus* from an overnight culture in MRS broth was spotted on the surface of Modified-MRS (M-MRS) agar (possessing 2% dextrose for decrease producing organic acid) and TSA, subsequently incubated at 37°C for 24 h to allow the development of colonies. After 24 h of culture, 50 μl of each of the pathogenic bacteria was inoculated in semi-solid TSA (TSB with yeast extract of 0.6% + agar 0.75%) and was poured over the M-MRS agars and TSA. The plates were incubated at 30°C for 24 h and checked for an inhibition zone. The inhibition zones were classified as (-) for no visible inhibition, (+) for 0.5 to 6 mm inhibition, (++) for 7 to 12 mm, and (+++) for more than 12 mm inhibition. (Perea Velez et al., 2007)

**Agar spot test with dead probiotic bacteria**

*L. rhamnosus* from an overnight culture in MRS broth was centrifuged at 5,000 g for 15 min. to remove the MRS broth. The *L. rhamnosus* cells were killed by 10% formalin for 30 min and washed with phosphate buffer saline (PBS) 3 times. The dead cells were spotted on the surface of M-MRS agar and overlaid with each pathogenic bacterium following the same method as above. The plates were incubated at 30°C for 24 h and checked for their inhibition zone.

**Disc diffusion assay**

Free cell supernatant was prepared from a 72 h culture of *L. rhamnosus* in M-MRS and MRS broth. Cells were removed by centrifuging at 5000 g for 15 min in

<table>
<thead>
<tr>
<th>Isolation No.</th>
<th>Pathogens</th>
<th>Host/organs</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. S. iniae I</td>
<td><em>S. iniae</em></td>
<td>fish/kidney</td>
<td>Japan</td>
</tr>
<tr>
<td>2. S. iniae II</td>
<td><em>S. iniae</em></td>
<td>fish/liver</td>
<td>Japan</td>
</tr>
<tr>
<td>3. S. iniae III</td>
<td><em>S. iniae</em></td>
<td>fish/brain</td>
<td>Thailand</td>
</tr>
<tr>
<td>4. S. iniae IV</td>
<td><em>S. iniae</em></td>
<td>fish/kidney</td>
<td>Thailand</td>
</tr>
<tr>
<td>5. S. agalactiae I</td>
<td><em>S. agalactiae</em></td>
<td>fish/kidney</td>
<td>Thailand</td>
</tr>
<tr>
<td>6. S. agalactiae II</td>
<td><em>S. agalactiae</em></td>
<td>fish/liver</td>
<td>Thailand</td>
</tr>
<tr>
<td>7. S. agalactiae III</td>
<td><em>S. agalactiae</em></td>
<td>fish/spleen</td>
<td>Thailand</td>
</tr>
<tr>
<td>8. A. hydrophila I</td>
<td><em>A. hydrophila</em></td>
<td>mixed breed frog/ blood</td>
<td>Thailand</td>
</tr>
<tr>
<td>9. A. hydrophila II</td>
<td><em>A. hydrophila</em></td>
<td>mixed breed frog/ liver</td>
<td>Thailand</td>
</tr>
<tr>
<td>10. A. hydrophila III</td>
<td><em>A. hydrophila</em></td>
<td>fish/spleen</td>
<td>Thailand</td>
</tr>
<tr>
<td>11. C. indologenes I</td>
<td><em>C. indologenes</em></td>
<td>mixed breed frog/ eyeball</td>
<td>Thailand</td>
</tr>
<tr>
<td>12. E. tarda I</td>
<td><em>E. tarda</em></td>
<td>fish/liver</td>
<td>Japan</td>
</tr>
</tbody>
</table>
sterile condition, the supernatant fluid was filtered through a filter with 0.22 μm pore size.

Five sterile blank paper discs were placed on the Muller Hilton agar which was inoculated with each pathogenic bacterium. Then, 100 μl of the filtered supernatants of L. rhamnosus were applied on the paper discs (Figure 1). Plates were incubated at 30°C for 24 h and observed for their inhibition zone.

**Growth inhibition by co-culture assay**

Pathogenic bacteria (*S. iniae* II, *S. agalactiae* I, *C. indologenes* I, *E. tarda* I, *A. hydrophila* II) were grown to lag phase in their suitable media. One hundred (100) μl (1x10⁷ CFU/ml) of each cultured pathogenic bacteria were inoculated in 10 ml of TSB with and without 100 μl (1x10⁷ CFU/ml) of cultured *L. rhamnosus*. After 24 h incubation at 37°C, the colony forming unit of each pathogenic bacterium and *L. rhamnosus* was counted by using MRS agar for *L. rhamnosus* and TSA for pathogenic bacteria. The results were expressed in percentage for each pathogen growth with *L. rhamnosus* by co-culture method compared with each pathogen growth without *L. rhamnosus* (control).

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>M-MRS inhibition zone</th>
<th>TSA inhibition zone</th>
<th>Disc diffusion assay</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. iniae</em> I</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>S. iniae</em> II</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td><em>S. iniae</em> III</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>S. iniae</em> IV</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>S. agalactiae</em> I</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>S. agalactiae</em> II</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>S. agalactiae</em> III</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>A. hydrophila</em> I</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td><em>A. hydrophila</em> II</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td><em>A. hydrophila</em> III</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>C. indologenes</em> I</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>E. tarda</em> I</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**Results**

**Agar spot test (Table 2):** By using agar spot test on M-MRS agar and TSA, *L. rhamnosus* inhibited the growth of all fish pathogens by producing a radius of at least 0.5 ml. For agar spot test on M-MRS agar the highest susceptible species was *A. hydrophila* I. The
highest susceptible species from the agar spot test on TSA was \textit{S. iniae} II. There was no inhibition zone of any bacteria from the agar spot test with killed probiotic bacteria.

**Disc diffusion assay:** Free cell supernatant from a 72 h culture of \textit{L. rhamnosus} in MRS broth inhibited growth of all fish and frog pathogens by producing a radius of at least 0.5 ml. All the results of disc diffusion assay are shown in Table 2. The highest susceptible species are \textit{S. iniae} II and \textit{A. hydrophila} I. When using supernatant from a 72 h culture of \textit{L. rhamnosus} in M-MRS broth with adjusted pH to 6.5 and untouched supernatant from a 72 h culture of \textit{L. rhamnosus} in M-MRS broth, there was no inhibition zone from either of them (Figure 3).

**Co-culture assay:** After having incubated each pathogen with probiotic bacterium for 24 h, the growth of each pathogen was lower than the control (Table 3). The lowest to the highest percent growth were as follows \textit{S. iniae} II, \textit{E. tarda} I, \textit{C. indologenes} I, \textit{A. hydrophila} II and \textit{S. agalactiae} I

**Discussion**

The present study on the agar spot test of \textit{L. rhamnosus} shows the efficacy of antimicrobial activity over all the aquatic pathogenic bacterial species used in this study. The agar spot test using killed probiotic bacteria, which showed no inhibition zones, clearly suggests that only the metabolic products, not the cells of probiotic bacteria, are involved in the growth inhibition of pathogenic bacteria. Lactic acid bacteria are known for their ability to produce inhibitory substrates such as hydrogen peroxide, organic acid and bacteriocin-like products, which are antimicrobials (De Vuyst and Leroy, 2007). The result of disc diffusion assay which showed...
the inhibition zone only when the disc was applied with the supernatant from MRS broth, may suggest that the main antimicrobial substance is the organic acid because when using supernatant from M-MRS broth (which cease producing of organic acid) there was no inhibition zone. In addition, the result from the agar spot test on TSA, which is not a suitable medium for lactic acid bacteria, might indicate that even in an unfavorable environment _L. rhamnosus_ still can produce antimicrobial substances. The potential for reducing the growth of pathogenic bacteria was confirmed by co-culture study. Here the probiotic and each pathogenic bacterium were cultured together in TSB. In all co-culture tubes the growth of pathogenic bacterium was lower when compared with the control tube in which just pathogenic bacteria were cultured. This is related to a previously study (Nikoskelainen et al., 2001). The mechanisms involved, are not only the antimicrobial substance that is produced from _L. rhamnosus_ and the change of pH in the co-culture tube, but also the ability of _L. rhamnosus_ in competition for nutrients (Verschueren et al., 2000).

From our study _L. rhamnosus_, a human-derived probiotic bacterium, is a promising probiotic candidate to be used in aquaculture with regard to its high potential against aquatic pathogenic bacteria _in vitro_. However, probiotic in aquaculture have many different properties from probiotics in humans. Information about the ability to adhere to host cells, their competitive exclusion stability in fish intestines, and the interaction between host-microbes _in vivo_ is further needed.

**Acknowledgement**

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**Reference**


Acute and Subacute Pulmonary Effects of Diesel Exhaust Particles in Mice: Pathological Changes and Translocation Pathways to the Circulation

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Abstract

To study the acute and subacute pulmonary effects of diesel exhaust particles (DEPs), mice were intratracheally instilled with 25, 50 or 100 μg of DEPs for dose response experiments. Histological alterations were determined at 3 days post-exposure. 50 or 100 μg of DEPs produced mild to moderate pulmonary inflammation and tissue injury characterized by infiltration of neutrophils and active alveolar macrophages (AMs), focal alveolitis and particle-laden AMs accumulation. Ultrastructural studies of treated animals showed the dissociation of basement membranes and erosion of type I alveolar epithelial cells. To investigate the time response, mice were instilled with 50 μg of DEPs and sacrificed at intervals from 1 to 30 days post-exposure. DEPs induced pulmonary inflammation and injury at acute period; however, these changes gradually regressed during the experiment. These results suggest that instillation of small doses of DEPs causes transient acute mild to moderate lung inflammation and tissue damage. The evidences of the DEPs distribution in lung tissues were also elucidated throughout the observation time. The main possible translocation pathway of DEPs from the lung to the circulation in this study could be cell mediated active transportation and direct penetration through the area of alveolar interstitial damages.

Keywords: acute, diesel exhaust particles, intratracheal instillation, pulmonary effects, subacute

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Introduction

Exposure to urban pollution, especially by particles, has been associated with a number of adverse health effects, including cardiopulmonary morbidity and mortality and lung cancer mortality (Dockery et al., 1993; Peters et al., 1999; Samet et al., 2000). In urban areas, diesel exhaust particles (DEPs) derived from diesel engine-powered automobiles are a major source of atmospheric PM2.5. DEPs are carbon-based particles that contain a variety of organic compounds such as polyaromatic hydrocarbons (PAHs), nitro-PAHs, quinines, heterocyclics, aldehydes, pyrenes and traces of heavy metals (Schuetzle, 1983; Schuetzle and Lewtas, 1986; Ghio et al., 2000). Previous studies showed that DEPs cause pulmonary inflammation and aggravate arterial and venous thrombosis (Mauderly et al., 1987; McClellan, 1987; Ichinose et al., 1995; Nemmar et al., 2003). In human, a short-term exposure to DEPs induces inflammatory responses in the airways and the peripheral blood (Salvi et al., 1999; Salvi et al., 2000). In vitro, DEPs are taken up by airway epithelial cells and stimulate the release of proinflammatory cytokines (Boland et al., 1999).
Comparison among health effect studies of DEPs can be complicated by variability in the chemical composition of the particles, which is influenced by the age and type of engine, fuel composition, load characteristics, lube oil components, presence and efficiency of emission control devices and sampling procedures (Schuetzle and Lewtas, 1986). Consequently, the biologic activities of sample generated and collected under different conditions are likely to be different.

Standard reference materials (SRMs) of DEPs (SRM 2975) that collected from an industrial diesel-powered forklift have been certified by the National Institute of Standards Technology (NIST, Gaithersburg, MD, USA) for use in evaluating analytical methods for the determination of selected PAHs in diesel particulate matter and similar matrices (Claxton et al., 1992). All of the chemical constituents provided in SRM 2975 are naturally present in particulate material. SRM 2975 was developed in part for mutagenicity assays (Hughes et al., 1997; DeMarini et al., 2004) and showed acute lung inflammatory responses on aspiration (Singh et al., 2004). However serial pathological changes in lung at acute and subacute stages are still not clarified.

Epidemiological studies have shown that the increase in mortality linked to particulate matter < 10 μm in diameter is attributable to cardiovascular functional disturbances and diseases (Pope et al., 1999). Although these clinical and epidemiological observations are strong and consistent, the underlying mechanisms responsible for the cardiovascular toxicity of particulate matter are still largely unknown. Several mechanisms responsible for the cardiovascular effects of particulate matters have been hypothesized. One of the hypotheses to explain these effects is that the particles cause inflammatory responses in the lung leading to release of mediators, which may influence the heart, coagulation, or other cardiovascular endpoints (Seaton et al., 1995). Another alternative hypothesis, which has not been much investigated so far, is that the particles translocate from the lungs into systemic circulation and thus influence hemostasis or cardiovascular integrity more directly. Studies suggest that PM2.5 have an important role in triggering biological responses and remain airborne for long periods of time. These particles can penetrate deeply into the respiratory tract and carry large amount of toxic compounds. A number of morphological studies have been demonstrated several translocation pathways of particulate particles (Takenaka et al., 2001; Shimada et al., 2006). However, there is no any morphological report on the translocation of DEPs from the lung to systemic circulation.

The purpose of this study is to describe acute and subacute pulmonary pathological effects caused by intratracheal exposure to DEPs (SRM 2975) and elucidate the possible translocation pathways from the lung into the systemic circulation using light and electron microscopy.

Materials and Methods

Particles: Standard reference materials (SRMs) of DEPs (SRM 2975) that collected from an industrial diesel-powered forklift have been certified by the National Institute of Standards Technology (NIST, Gaithersburg, MD, USA). According to NIST analyses, SRM 2975 samples consist of polycyclic aromatic hydrocarbons (PAHs) and 1-nitropyrene compounds. The scanning electron microscopic images of SRM 2975 showed fine polygonal particles with diameters approximately < 10 μm. The specific surface area, which measured by Brunauer, Emmett and Teller (BET), was 91 m²/g.

Experimental design

Dose response: To find the appropriate dose for determination of DEPs lung toxicity, the doses of 25, 50 or 100 μg of SRM 2975 were used. Forty-eight male ICR mice were randomly separated into four control and twelve exposure groups of 3 animals each. Twelve exposure groups were single intratracheally instilled with 50 μl aqueous suspensions of 25, 50 or 100 μg of SRM 2975 suspended in 0.01 M phosphate-buffered saline (PBS). The four groups of control were instilled with 50 μl of 0.01 M PBS. At 5 min, 6, 24, and 72 hr after instillation, the animals in each group were sacrificed and various organs such as lung, hilar lymph node, heart, liver and kidney were collected and preserved in 10% buffered neutral formalin for routine histopathological evaluations and 2.5% glutaraldehyde for electron microscopic examinations.

Time effect: Seventy mice were divided randomly into 30 groups of 2-3 animals each. The fifteen treated groups were single intratracheally instilled with 50 μl aqueous suspensions of 50 μg of SRM 2975 suspended in 0.01 M
phosphate-buffered saline (PBS). The rest of fifteen control groups of mice were instilled with 50 μl of 0.01 M PBS. Animals in each group were sacrificed at 0, 5, 10, 30 min, 1, 2, 6, 12, 24, 48, 72 hr, 5, 7, 15, 30 days post-exposure, respectively. The tissue samples were collected as described above.

**Ultrastructural studies:** Using transmission electron microscope, lung tissue samples were post-fixed in 1% osmium tetroxide, dehydrated with serial alcohol and embedded in epoxy resin. Ultrathin sections stained with uranyl acetate and lead citrate were examined with a JEM-100CX electron microscope (JEOL, Tokyo, Japan).

**Results**

**Dose responses of SRM 2975**

**Clinical and gross findings:** In control and all of treated animals, there were no exposure-related clinical signs in any observation time. Grossly, instillation of 50 and 100 μg of DEPs caused mild congestion and edema in the lungs compared to 25 μg treated and control groups.

**Histopathology:** No significant lesions were observed from the lungs of control animals at all observed times. However, accumulation of free aggregated particles was found in the alveoli and bronchiolar lumens of all treated groups at 5 min after instillation. Some of aggregated particles were present within alveolar macrophages, and occasionally present within alveolar epithelial cells (Figure 1A). Furthermore, increasing number of cells in alveolar wall was also observed in the lungs of all treated groups. By 6 hr after instillation, the animals instilled with 50 and 100 μg DEPs had mild to moderate influx of neutrophils into the alveolar air spaces and into the interstitium around peribronchiolar vessels (Figure 1B). At 24 hr after the instillation, a number of nodular aggregate of neutrophils and particle-laden AMs were observed in some alveolar regions particularly in the perivascular areas adjacent to the bronchioles. By 3 days after instillation, mild to moderate focal alveolitis characterized by accumulation of numerous active AMs, particle-laden AMs, and some neutrophils were observed.

![Figure 1A](image1a.png)

![Figure 1B](image1b.png)

![Figure 1C](image1c.png)

**Figure 1.** Lung tissues from various doses of DEPs treated groups show variety of pulmonary effects, H&E stain. (A) the distributions of DEPs in AMs (arrow) and alveolar epithelium (arrowhead); 25 μg treated group, (bar = 500 μm). (B) Influx of neutrophils around the DEPs accumulation; 100 μg treated group, (bar = 40 μm). (C) Focal alveolitis; 100 μg treated group, (bar = 700 μm).
at the terminal bronchiolar and alveolar duct regions (Figure 1C). The hilar lymph nodes of all treated groups were slightly enlarged with some particle-laden AMs infiltration into lymphoid parenchyma. The lesions of lungs and lymph nodes in mice treated with DEPs at the dose of 50 and 100 μg were similar in appearance but the magnitude of these appearances were greater in mice treated with 100 μg DEPs than in mice treated with 50 μg. In 25 μg DEPs treated groups showed only similar histopathological patterns. However, the lesions were milder and occupied a small area of the lung specimens.

**Transmission Electron microscopy:** Increased numbers of pinocytic vesicles in alveolar type I cells and endothelial cells of lungs from DEPs treated animals was observed at the area, where the aggregation of DEPs was also present. In 50 and 100 μg DEPs treated groups, type I alveolar epithelial cells that apposed to the clump of particles had features of necrosis and desquamation leading to direct exposure of interstitial tissue to the alveolar space. Damage and dissociation of basement membranes were also found associated with type I alveolar epithelial cell erosion from 5 min onwards (Figure 2).

**Time effects of SRM 2975**

**Histopathology:** In the control groups, no significant lesion was observed at all time points. At the acute stages of experiment (from 0 hr to 7 days post-exposure), free singlet and aggregate forms of particles were observed on the bronchiolar epithelial cell surface and along the apical surface of the plasma membrane of alveoli. Many singlet DEPs were seen in alveolar epithelial cell cytoplasm, in which some particles were occasionally observed in alveolar capillary lumens even at 0 min post-exposure. Increasing number of active and particle-laden alveolar macrophages was seen scattering around the accumulation of particles in alveoli and bronchioles. Increasing of cells in alveolar septal walls was also found in the area related to DEPs accumulation. At 12 and 24 hr after instillation, a number of nodular aggregations consisted of neutrophils, active AMs, particle-laden AMs and some cell debris were observed in some alveolar regions adjacent to the

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**Figure 2.** An electron micrograph of the lung from treated mouse revealed desquamation of necrotic alveolar type I cells (solid arrow) with aggregation of DEPs particles; 50 μg treated group, TEM, bar = 2.4 μm. Aa: alveolar air space, BM: basement membrane, ET: alveolar endothelial cell, RBC: red blood cell, Type I: type I alveolar epithelial cell, P: DEPs particles.

**Figure 3.** Photomicrographs from the lungs of 50 μg DEPs-treated mice sacrificed at various time points. H&E stain. (A) Treated mice sacrificed at 1 day after exposure showed moderate infiltration of neutrophils with an inflammatory nodule in alveolar air spaces. (bar = 700 μm). (B) Mice sacrificed at 3 days after instillation with DEPs showed moderate focal alveolitis consisting of numerous active AMs, particle-laden AMs, lymphocytes and fewer neutrophils. (bar = 700 μm).
bronchioles (Figure 3A). Particle-laden AMs and neutrophils infiltration into bronchial associated lymphoid tissue (BALT) was also observed. By 48 and 72 hr after instillation, moderate focal alveolitis characterized by accumulation of numerous active AMs, particle-laden AMs, lymphocytes and some neutrophils was observed at the terminal bronchiolar and alveolar duct regions (Figure 3B). Changes in the lungs of mice killed at 5 and 7 days after DEPs instillation were restricted to the appearance of the aggregated small foci consisting of particle-laden AMs, active AMs, lymphocytes and fibroblasts with occasional type II epithelial cell regenerative hyperplasia. The hilar lymph nodes were slightly enlarged associated with accumulation of particle-laden macrophages and hyperplastic histiocytes in subcapsular and medullary sinuses. At the subacute stages (from 15 to 30 days post-exposure), the inflammatory foci in lung parenchyma were reduced in number and more focally concentrated.

Transmission Electron microscopy: Numerous free particles, singlet and aggregate forms, of DEPs were observed on the bronchiolar epithelial cell surface and along the apical surface of the plasma membrane of alveoli in treated animals. Accumulation of particle-laden AMs was also found in the alveoli and bronchiolar lumens even at 24 hr post-exposure. These particle-laden AMs showed round or polygonal shapes with numerous lysosomes and phagolysosomes containing particles (Figure 4A). Instilled particles were also observed in the cytoplasm of both type I and II alveolar epithelial cells (Figure 4B). Increasing number of pinocytic vesicles with some particle-containing in type I alveolar epithelial cells and capillary endothelium was also observed. Necrosis and desquamation of type I alveolar epithelial cells were found particularly in the areas apposing to the clump of DEPs leading to direct exposure of interstitial tissue to the alveolar space. Damage and dissociation of basement membranes were also found associated with type I alveolar epithelial cell erosion.

Discussion

To estimate the suitable doses based on dose-dependent toxicity, we exposed mice with a single intratracheal instillation of various low doses of DEPs. Our data showed that even a low dose (25 μg/mouse) of DEPs can induce pulmonary effects characterized by mild inflammatory cell infiltration, mainly alveolar macrophages and neutrophils, increasing number of cells in alveolar wall and particle-laden AMs accumulation. By contrast, instillation of higher doses (50 or 100 μg) DEPs produced mild to moderate pulmonary inflammation and injury, as evidenced by infiltration of neutrophils and active AMs, focal alveolitis and particle-laden AMs accumulation. Ultrastructural studies in treated animals showed the dissociation of basement membranes and erosion of type I alveolar epithelial cells, which were associated with particle accumulation. These pathological
appearances suggested that DEPs can induce alterations in the permeability of the alveolar-capillary barrier resulted in the disturbance of leakage of the transudation of serum proteins from the vasculature into alveolar lumens similar to the previous study (Singh et al., 2004). From the results of dose response experiments in recent study, 50 μg was suggested to be the most appropriate dose for sensitive detection of acute and subacute inflammatory changes in lungs exposed to DEPs. The dose of 100 μg did not induce any novel extra alterations with a little bit too large to allow assessment of slight changes and the distribution of particles in lung parenchyma. In addition, 25 μg was too weak to cause changes in histological parameters on subacute stage. Ichinose (1995) reported that intratracheal instillation of 400 or 800 μg DEPs per mouse at 24 hr post-exposure produced a moderate to marked pulmonary inflammation and injury effects characterized by influx of neutrophils, accumulation of particle-laden alveolar macrophages, and alveolar type I cell and basement membrane damages that quite similar to our histological changes at acute phase but caused much more severe lesions because of large volume of particles.

The results of time effects showed that instillation of low dose of DEPs (50 μg) induced mild to moderate pulmonary inflammation and injury characterized by infiltration of neutrophils and active AMs, focal alveolitis and particle-laden AMs accumulation at subacute stage. However, the lung lesions with occasional regenerative hyperplasia of type II epithelial cells were milder during subacute phase. Ultrastructural finding showed the dissociation of basement membranes in alveolar area related with the particle accumulation, and then gradually recovered to normal appearances. Chronologically, the lung lesions were getting better by time because of effective clearance system against toxic effects associated with particle exposure. The clearance pathways of insoluble particles deposited in the alveolar region involves phagocytosis by alveolar macrophages followed by mucociliary clearance system and through the lymphatic system (Sun et al., 1984; Cohen et al., 1985; Yu and Rappaport, 1996). From the histological results, we suggested that the most prominent alveolar clearance pathway in our study was mediated by alveolar macrophages, primarily through migration of particle laden cells to the ciliated airways, and to a small portion through penetration of these cells into the interstitium where they were either retained or transported to the lymphatic system.

There are three hypothetical mechanisms of the particulate matter translocation at the air-blood barrier including cell mediated active transportation (phagocytosis by macrophages or endocytosis by alveolar epithelial cells), passive transportation (diffusion pathway), active or passive transportation through gaps between alveolar epithelial cells (Heckel et al., 2004; Oberdorster et al., 2005). From the results of our ultrastructural study, DEPs could translocate through air-blood barriers to the circulation by cell mediated active transportation, even phagocytosis by macrophages or endocytosis by alveolar epithelial cells. Moreover, damage of alveolar type I cells and basement membranes caused by exposure to DEPs enhance the opportunity of particles to penetrate directly into the blood circulation.

In summary, this study showed pathological details of acute pulmonary inflammation and tissue injury induced by intratracheal instillation of various low doses of DEPs. Dose-response pulmonary effect of DEPs (SRM 2975) was also revealed. In the time effect study, the main translocation of DEPs (SRM 2975) might be translocate through air-blood barriers to the circulation by cell mediated active transportation, either phagocytosis by macrophages or endocytosis by alveolar epithelial cells. Moreover, alveolar basement membrane damages caused by DEPs might be one of the causes of the translocation.

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References


Efficacy of Various Strains of Infectious Bronchitis Vaccine against Nephropathogenic Infectious Bronchitis Virus Isolated from Chickens in Thailand

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Abstract

One hundred, 1 week old, female broiler chickens were divided into 5 groups, 20 chickens in each. Each group was vaccinated with a different strain of infectious bronchitis vaccine; Ma5, H120 and a local strain produced by the Department of Livestock Development for groups 3, 4 and 5, respectively. All the vaccinated chickens received intraocular vaccine at 1 week old. The chickens in groups 1 and 2 served as a negative control and a positive control group, respectively. All the chickens were weighed at 1, 4 and 6 weeks old and challenged at 4 weeks old with nephropathogenic infectious bronchitis virus isolate THA001. Clinical signs and mortality rates were observed for 2 weeks post-challenge. Serological responses were determined at 1, 2, 3, 4, 5 and 6 weeks old. The results revealed that the body weight of chickens among the vaccinated groups was not significantly different \((p>0.05)\) both before and after challenge but the body weight of chickens in the vaccinated group was significantly higher than the chickens in the positive control group \((p<0.05)\). After challenge, the mortality rate of the chickens in groups 3, 4 and 5 was significantly lower than that of the positive control group \((p<0.05)\). The serological response of chickens in the vaccinated groups at 2 weeks post-challenge was significantly higher than that of the positive control group \((p<0.05)\).

Keywords : broiler chickens, nephropathogenic infectious bronchitis virus, vaccine

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Introduction

Infectious bronchitis (IB) caused by infectious bronchitis virus (IBV) is a common health problem in poultry that has a high economic impact especially in the chicken industry in most countries of the world. The IBV can damage the respiratory tract in chickens of all ages (Parson et al., 1992). The severity of the disease varies by age. In young chickens, symptoms are more severe and the mortality rate is higher when compared to adult chickens (Animas et al., 1994). Infected chickens show a decrease in growth rate and an increased in feed conversion ratio. The disease is a risk factor for secondary bacterial infections of the respiratory tract resulting in an even higher morbidity and mortality rate (Ziegler et al., 2002). The decrease in egg production can be up to 30% in infected layers (Gough et al., 1992). Nephropathogenic infectious bronchitis (NIB) strains cause lesions in the kidneys; for example, nephritis, enlarged kidneys, pale kidneys and accumulated urate in the ureter (Ziegler et al., 2002).

The occurrence of NIB has been reported in many countries. The mortality rate of infected chickens with NIB is 23-30% (Ziegler et al., 2002). In Thailand, NIB is an endemic disease that can be found all over the country. Upatoom et al. (1983) reported incidences in the north-east of Thailand in broilers aged 14-28 days with 1-15% mortality rate. Among other reports were, 35 incidences in the south of Thailand, 29 in broilers aged 12-44 days, 5 in layers aged 8-40 weeks and 1 in 3 months old native chickens. Respiratory symptoms along with enlarged kidneys were observed in 21 incidences and enlarged kidneys only were observed in 4 incidences (Antarasena et al., 1990).

Vaccination for the prevention of IB is widely practiced. However, NIB has been isolated in vaccinated chickens. The vaccine failure may be caused by the differences in the genotypes or serotypes of vaccine strains and field strains (Pensaert and Lambrechts, 1994). In some cases, improvements in protection might be achieved by the use of a different IB vaccination (Fabio et al., 2000). At present, many strains of vaccine are commercially available. It is important to periodically evaluate the cross-protective capabilities of vaccines versus field isolates, because the outcomes of these studies will provide valuable information on the practical use of existing vaccines and the potential need for new ones. The objective of this study was to evaluate the efficacy of vaccines using Ma5, H120 and a local strain produced by the Department of Livestock Development (DLD) against NIB isolated from chickens in Thailand.
Materials and Methods

Virus propagation and titration: The viral agent, isolate THA001 (accession number DQ449628), was isolated from an infected chicken with respiratory and renal lesions in central part of Thailand in 1998 and preserved in allantoic fluid at -70°C. The preserved agent was thawed and then propagated by inoculation in embryonated chicken eggs via the allantoic cavity. The eggs were incubated at 37°C for 72 hours before being stored at 4°C for 12-18 hours. Then the allantoic fluid was collected and frozen at -70°C for stock solution.

Virus titration: Determination of the virus concentration in the stock solution was done by the 10 fold serial dilution method. Six, 10 days old embryonated chicken eggs were inoculated in the allantoic cavity with 0.1 ml per egg of each tenfold serial dilution of the virus and kept at 37°C. Seven days after the inoculation, the embryos were examined for IBV lesions (stunting, curled toes or urates in the mesonephrons) (Ziegler et al., 2002). The control embryonated eggs have no those lesions at the same period. The embryo infectious dose 50% (EID<sub>50</sub>/0.1ml) was calculated according to Reed and Muench (1938).

Experimental design: One hundred, 1 week old female broiler chickens were randomly allocated into 5 groups with 20 chickens each and housed in separate experimental rooms. Feed and water were supplied ad lib. An infectious bronchitis vaccine was administered to the chickens via eye drops. Three kinds of commercial vaccines including Ma5 (Intervet, Holland), H120 (Intervet, Holland) and a local strain (DLD, Thailand) were used for vaccination.

The groups were treated with the following regime; no infection and no vaccination (group 1), infection and no vaccination (group 2), infection and vaccination with Ma5 strain (group 3), infection and vaccination with H120 strain (group 4), infection and vaccination with local strain produced by DLD (group 5). The guidelines and legislative regulations on the use of animals for scientific purposes of Chulalongkorn University, Bangkok Thailand were followed as is certified in permission no. 16/2548.

Challenge inoculation: Three weeks after the vaccination, the chickens in groups 2-5 were inoculated with 100 μl of IBV via eye drops. The virus concentration was approximately 10<sup>6.5</sup> EID<sub>50</sub>.

Clinical signs, mortality and body weight gains: Clinical signs and mortality were observed and recorded for 2 weeks after the challenge. Necropsy was done on the carcasses for gross pathologic lesions. The chickens were weighed before the vaccination, the day before the challenge and at the end of the experiment.

Detection of IBV antibody titers: Before the vaccination, blood samples were randomly collected from 10 chickens. Then, blood samples were randomly collected from 10 chickens in each group every week until the sixth week. The serum samples were preserved at -20°C. IBV antibody titer was monitored using commercial Enzyme Linked Immunosorbent Assay (ELISA) test kit (BioChek, Holland).

Statistical Analysis: A comparison of body weight and antibody titer among the experimental groups was done using ANOVA and the Duncan’s Multiple Range test. The mortality rate among the groups was compared by Chi-square test.

Results

Clinical signs and mortality rate: After the challenge inoculation, infected chickens showed signs of respiratory disorders; i.e. nasal and ocular discharge, coughing, sniffing, mouth breathing and respiratory distress. Clinical signs of the gastrointestinal system observed included watery diarrhea and contamination of urate in feces. The mortality rate was 35% in group 2, which was infected but was not vaccinated, and the mortality rate was 5, 0, and 5% in groups 3, 4, and 5, respectively (Table 1). The mortality rate of group 2 significantly differed from those of the other groups (p<0.05). Necropsy revealed that all the carcasses contained lesion in the trachea and the kidneys. Mucus accumulated in the trachea, enlarged kidneys and an accumulation of urate in the ureter were observed in the necropsied chickens.

Body weight of chickens: The body weight of the chickens in each group did not significantly differ at 1 and 4 weeks old (p>0.05). At 6 weeks old, 2 weeks after challenge inoculation, the average body weight of the chickens in group 2 was significantly less than the other groups (p<0.05) but no significant difference to the average body weight was found among the other groups (p>0.05) (Table 1).

IBV Antibody titers: At 2 weeks old, the average IBV antibody titer of all groups was not significantly different. At 3 weeks old, the chickens in group 4 had the highest average IBV antibody titer and it was significantly different from the others (p<0.05). At 4 and 5 weeks old, the chickens in group 2 had the lowest average IBV antibody titer and it was significantly different from groups 3 and 4 (p<0.05) but not significantly different from group 5 (p>0.05). Interestingly, the average IBV antibody titer at 5 weeks old of all the vaccinated groups was distinctly elevated above the unvaccinated group (Figure 1). At the end of experiment (6 weeks old), the chickens in groups 3, 4 and 5 had significantly higher the average IBV antibody titers than the chickens in group 2 (p<0.05). No chickens from group 1 (negative control)
were positive for IBV antibodies during the time of the experiment.

**Discussion**

The mortality rate of the chickens that were vaccinated (0-5%) was lower when compared to infected chickens that were not vaccinated (35%), indicated that the vaccines used in this study could induce cross protection against the field challenge strain that causes NIB. These results are consistent with other studies that show cross protection between different strains of vaccine. Darbyshire (1985) reported that the H120 strain vaccine was effective against the Australian T strain of IBV, which was able to cause renal lesions. The vaccine could reduce the amount of agent 30,000 times within 4 days of infection. Wang et al. (1996) evaluated the efficacy of the H120 strain vaccine by observing the movement of cilia in the trachea and renal lesions caused by 1171 and 1449 strains and found that only 1 in 10 chickens that had received the 1171 strain did not show signs of infection. Albassam et al. (1986) showed that chickens that had received the H120 strain vaccine at 10 days old and were infected 4 weeks later with strains that caused renal lesions; Gray, Holte, Australian T and Italian, did not show tracheal lesions but 50-70% of the chickens showed renal lesions. This indicated partial protection of the vaccine across the strains. It has been known that the trachea is a primary site for the IBV to propagate. Live vaccines can reduce mortality in infected animals due to mucosal immunity induced by the virus. The mucosal immunity prohibits invasion and propagation of the virus in the tracheal mucosa. When the vaccine strain is different from the strain that causes infection, some infectious agents overcome the immune mechanism and invade the kidneys resulting in morbidity which is less severe (Pensaert and Lambrechts, 1994). Thompson et al. (1997) examined the mucosal immunity of infected chickens and found that 70% of the samples contained IgA, 52% and 56% of the samples contained IgG and IgM, respectively. Nakamura et al.

**Table 1**  Body weight and mortality rate of the chickens in each group

<table>
<thead>
<tr>
<th>Group</th>
<th>Vaccination</th>
<th>Body Weight (gram±SD)</th>
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<td></td>
<td></td>
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<td>4 weeks old</td>
</tr>
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<td>178.3±11.3 a</td>
<td>1223.0±67.4 a</td>
</tr>
<tr>
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<td>positive control</td>
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</tr>
<tr>
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<td>H120</td>
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<td>1262.5±70.7 a</td>
</tr>
<tr>
<td>5</td>
<td>DLD</td>
<td>178.3±11.7 a</td>
<td>1268.5±84.3 a</td>
</tr>
</tbody>
</table>

Different superscripts in the same column indicate significant difference ($p<0.05$)

**Figure 1**  The average IBV antibody titers of the chickens in each group

![IBV antibody titer](image)
(1991) found IgM, IgA, and IgG in the trachea more often in chickens that were resistant to the disease compared to susceptible chickens. Live vaccines can also induce cellular immunity, which can prohibits virus attack as well. Pei et al. (2003) found that transfer of CD8+ T cell isolated during 3-6 weeks after infection caused by IBV to 6 days old chicks can protect the chicks from infection.

The average antibody titer of vaccinated chickens after the challenge was higher than those of unvaccinated chickens due to recognition of the virus by the immune system (Thompson et al., 1997). There were 11 sites (epitopes) of spike glycoprotein, which is located on the envelope of the infectious bronchitis virus, that are specific to antibodies. These epitopes are 7 type specific epitopes and 4 group specific epitopes (Parr and Collisson, 1993). The cross protection observed in this study may be caused by group specific epitopes that stimulate the immune response to the challenge virus. At 6 weeks old, body weight and mortality rate of vaccinated chickens, which received the challenge virus, and the negative control chickens were not different but they were significantly better than those of the positive control chickens. The results indicate the protective efficacy of all tested vaccines, namely Ma5, H120 and DLD against the local NIB strain.

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References


Detection of Antibody Titer Against *Toxoplasma gondii* in Aborted Sows and Gilts in a Swine Herd in Thailand

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Abstract

The objective of the present study was to investigate the sero-prevalence of *Toxoplasma gondii* (*T. gondii*) among aborted sows and gilts in a commercial swine herd in Thailand. Data were collected from a swine breeding herd in Thailand between January and November 2008. Abortion rate was increased from 2.2% in January-June 2008 to 9.3% in July-October 2008. Among 132 aborted sows and gilts observed between July to October 2008, the average abortion date was 50.4 days gestation. During abortion, chlortetracycline 600-800 ppm was medicated in sow feed in September for 4 weeks (600 ppm for 2 weeks and 800 ppm for 2 weeks). In October, sulfa-trimetroprim 500 ppm was added to the feed. The number of aborted sows was dramatically reduced in November. Reproductive performance of sows greatly declined after the abortion epidemic. For instance, from March to October, the farrowing rate decreased from 86.7% to 65.6%, the number of piglets born alive per litter decreased from 10.4 to 8.6 piglets/litter and the percentage of mummified fetuses increased from 3.3% to 9.3%. Blood samples were randomly collected from aborted gilts and sows in September and October and these were analyzed for antibody titer for Swine Fever, Aujezkey’s Disease, Porcine Parvovirus infection, Porcine Reproductive and Respiratory Syndrome (PRRS), Leptospirosis and Toxoplasmosis. *T. gondii* infection was analyzed using latex agglutination test. Antibody against Swine Fever among aborted sows varied from 1:16 to 1:32. All of the aborted females were negative for Aujezkey’s Disease. Antibody titer against Porcine Parvovirus varied from 1:64 to >1:4096. Two out of three aborted sows were sero-negative for PRRS. Of the aborted gilts and sows, 12 out of 13 tested sows (92.3%) were *T. gondii* sero-positive with a titer between 1:64 and 1:128.

**Keywords**: abortion, antibody, latex agglutination test, pig, *Toxoplasma gondii*.

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Introduction

Toxoplasmosis is one of the most common zoonotic diseases caused by an obligate intracellular protozoan parasite Toxoplasma gondii (T. gondii) (Dubey 1986; Suaréz-Aranda et al. 2000; Damriyasa et al. 2004; Venturini et al. 2004; Kijlstra et al. 2008). The disease causes abortion and stillbirth in pregnant women, pregnant sows and several mammalian species (Venturini et al. 1999). Felids serve as a definitive host by shedding oocysts into the feces whereas a wide range of warm-blooded animals serve as an intermediate host by harboring tissue cysts in the brain, eye, skeletal muscle, and cardiac muscle. Three infectious stages of T. gondii responsible for disease transmission include tachyzoite, bradyzoite in tissue cyst, and sporozoite-containing oocyst (reviewed by Dubey 2004). The infection can be acquired orally by ingestion of oocyst-contaminated water or food and ingestion of undercooked cyst-containing meat. Congenital infection occurs transplacentally resulting in abortion, stillbirth, birth defect,
chorioretinitis and mental retardation. In humans, primary *T. gondii* infection during pregnancy can lead to infection of the fetus, hence causing fetal death (reviewed by Dubey 2004).

*T. gondii*-infected pigs are an important source of transmission to humans due to the consumption of undercooked pork (Dubey et al. 2005). The prevalence of *T. gondii* varied considerably in pigs depending on such factors as herd management, housing, age group and herd location (Suárez-Aranda et al. 2000; Venturini et al. 2004). Seropositive pigs harbour tissue cysts in their meat after being naturally infected with *T. gondii*. The introduction of *T. gondii* into the pig farm increases the risk of infection in pregnant gilts and sows. Rodents are considered as a reservoir host and play an important role in the transmission of the disease to pigs and humans. Consequently, intensive control of rodents can significantly reduce the prevalence of *T. gondii* in pig herds (Kijlstra et al. 2008). In modern pig farms, *T. gondii* has nearly been eliminated in some countries (Kijlstra et al., 2008; Poljak et al., 2008). Nevertheless, several countries reported the prevalence of this infection (Suárez-Aranda et al. 2000; Venturini et al. 2004; de Sousa et al. 2006). However, little information on *T. gondii* in pigs in Thailand is available.

In this study, we investigated the sero-prevalence of *T. gondii* infection among aborted sows in a commercial swine herd in Thailand. This report aimed to reveal the occurrence of *T. gondii* in a pig farm in order to explore the need for increased attention to monitor *T. gondii* infection in pigs, and enhanced public health concern about *T. gondii* in Thailand.

**Material and Methods**

**Herd and general management:** This study was conducted in a swine breeding herd in Thailand during January to November 2008. The number of sow inventory was 1,602 sows. Data from a total of 132 aborted gilts/sows were included (data from these aborted females were collected from July to October). Reproductive data from a total of 3,548 Landrace x Yorkshire crossbred gilts/sows inseminated during January to November 2008 were collected. The gilts and sows were housed in a conventional open housing system equipped with water sprinklers and fans, whereas the boars were kept in an evaporative cooling system. The health of the herd was monitored by the herd veterinarian. The vaccination against Foot-and-Mouth Disease (FMD), Swine Fever (SF), Aujeszky’s Disease (AD), Porcine Parvovirus (PPV) and Atrophic Rhinitis (AR) have been performed. The gilts were mated at >32 wk of age with a body weight of >135 kg at the second or later observed oestrus. The mating technique was performed by conventional artificial insemination. The gilts and sows received water up to *ad libitum*. The feed was provided twice a day with a corn-soybean-fish base containing 15-18% crude protein, 3,000-3,200 kcal/kg ME and 0.9-1.0% lysine.

**Blood sampling and serological testing:** Blood samples were collected from the jugular vein of 30 aborted gilts and sows. Blood samples were allowed to clot at room temperature, and sera were obtained and kept at -20°C for analyzing antibody titer against SF (n=10), AD (n=10), PPV (n=10), Porcine Reproductive and Respiratory Syndrome (PRRS) (n=4), Leptospirosis (n=17) and *Toxoplasma gondii* (n=13). Antibody against gI of AD virus was determined using HerdChek® Anti-PRV gpl test kit (IDEXX Laboratories, Inc., USA). PPV was determined by using haemagglutination inhibition (HI) test. PRRS virus was determined using HerdChek® PRRS virus antibody test kit 2XR (IDEXX Laboratories, Inc., USA). The serum sample/positive control (S/P) ratio below 0.4 indicated that the sample had no antibody against PRRS virus (negative), while S/P ratio > 0.4 indicated that the sample had antibody against PRRS virus (positive). In addition, PRRS virus from pooled serum was detected using the reverse transcriptase-polymerase chain reaction (RT-PCR) technique. Antibody response to *T. gondii* infection was analyzed using the latex agglutination test (TOXOREAGENT ‘Eiken’,
Eiken chemical Co. Ltd., Tokyo, Japan) (Tsubota et al., 1977). The test was performed according to the manufacturer’s instruction. A positive result was defined as serum with titer ≥1:64.

Statistical analysis: The statistical analysis was performed using SAS (SAS version 9.0, Cary NC, USA.). Descriptive statistics were performed for quantitative data. Qualitative data were evaluated using frequency analysis and \( r \times k \) contingency table.

**Results**

The aborted sows, aborted fetuses, the herd’s environment and the presence of rats and cats in the herd are shown in Figure 1. On average, the number of gilts and sows inseminated monthly was 354.7±21.8 gilts/sows (range 328-394 gilts/sows). From January to June 2008, the number of aborted females was 48 gilts/sows (approximately 8 females per month), given an estimated abortion rate of 2.2% \((8/354.7) \times 100\) monthly. The number of gilts and sows aborted in July, August, September, October and November, 2008 was 16, 28, 34, 54 and 17 gilts/sows, respectively. The estimated abortion rates were 4.5%, 7.9%, 9.6%, 15.2% and 4.8% in July, August, September, October and November, 2008, respectively. During July to October, the overall abortion rate was 9.3%. The numbers of aborted fetuses from each female were not recorded. Feed was given to the gestating gilts and sows twice daily. Normally, the feed was stored in a modified semi-automatic feeder in the barn overnight before feeding to the pregnant sows early in the morning. Cats and rats were commonly found in this herd, particularly during night time.

The antibody titers of aborted gilts and sows against AD (gI antibody), SF and PPV are presented in Table 1. Of these aborted gilts and sows, antibody titer against *Leptospira serova Shermani* (1:40) was found in 8 out of 10 samples, and *Leptospira serova Ranarum* (1:40) was found in 4 out of 7 samples. Sero-positive PRRS was found in one aborted sow (S/P ratio 0.987). Two out of three aborted sows were sero-negative for PRRS (S/P ratio <0.4). The analyses of RT-PCR for the PRRS virus revealed that all sera from aborted sows and a boar were negative. Of the 13 aborted sows, 12 sows (92.3%) were *T. gondii* sero-positive for the latex agglutination test with a titer between 1:64 and 1:128 (Table 2).

**Table 1** Antibody titers of aborted gilts and sows against Aujezkey’s disease (AD, gI antibody), Swine Fever (SF) and Porcine Parvovirus (PPV)

<table>
<thead>
<tr>
<th>Number</th>
<th>Parity</th>
<th>Interval from abortion to blood sampling</th>
<th>AD</th>
<th>SF</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gilt</td>
<td>1</td>
<td>Negative</td>
<td>1:16</td>
<td>&gt;1:4096</td>
</tr>
<tr>
<td>2</td>
<td>Gilt</td>
<td>1</td>
<td>Negative</td>
<td>1:16</td>
<td>&gt;1:4096</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>6</td>
<td>Negative</td>
<td>1:32</td>
<td>&gt;1:4096</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>7</td>
<td>Negative</td>
<td>1:32</td>
<td>1:64</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>7</td>
<td>Negative</td>
<td>1:32</td>
<td>&gt;1:4096</td>
</tr>
<tr>
<td>6</td>
<td>Gilt</td>
<td>16</td>
<td>Negative</td>
<td>1:16</td>
<td>1:2048</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>4</td>
<td>Negative</td>
<td>1:16</td>
<td>&gt;1:4096</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>4</td>
<td>Negative</td>
<td>1:16</td>
<td>&gt;1:4096</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>10</td>
<td>Negative</td>
<td>1:16</td>
<td>1:128</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>6</td>
<td>Negative</td>
<td>1:32</td>
<td>1:512</td>
</tr>
</tbody>
</table>
Table 2  Antibody titers, period of gestation and interval from abortion to blood collection from 13 aborted sows

<table>
<thead>
<tr>
<th>Number</th>
<th>Gestation (d)</th>
<th>Interval from abortion to blood sampling</th>
<th>Toxoplasma gondii</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>18</td>
<td>Negative</td>
</tr>
<tr>
<td>2</td>
<td>65</td>
<td>34</td>
<td>Positive (1:128)</td>
</tr>
<tr>
<td>3</td>
<td>69</td>
<td>15</td>
<td>Positive (1:128)</td>
</tr>
<tr>
<td>4</td>
<td>62</td>
<td>19</td>
<td>Positive (1:128)</td>
</tr>
<tr>
<td>5</td>
<td>60</td>
<td>23</td>
<td>Positive (1:128)</td>
</tr>
<tr>
<td>6</td>
<td>85</td>
<td>7</td>
<td>Positive (1:128)</td>
</tr>
<tr>
<td>7</td>
<td>57</td>
<td>20</td>
<td>Positive (1:128)</td>
</tr>
<tr>
<td>8</td>
<td>27</td>
<td>22</td>
<td>Positive (1:64)</td>
</tr>
<tr>
<td>9</td>
<td>32</td>
<td>7</td>
<td>Positive (1:64)</td>
</tr>
<tr>
<td>10</td>
<td>74</td>
<td>1</td>
<td>Positive (1:128)</td>
</tr>
<tr>
<td>11</td>
<td>36</td>
<td>1</td>
<td>Positive (1:128)</td>
</tr>
<tr>
<td>12</td>
<td>49</td>
<td>1</td>
<td>Positive (1:128)</td>
</tr>
<tr>
<td>13</td>
<td>NA</td>
<td>NA</td>
<td>Positive (1:128)</td>
</tr>
</tbody>
</table>

NA: data not available

Figure 1  Herd environment and management, the presence of a cat in the pig herd (a) semi-automatic feeding (b) a dead rat (c) and aborted fetuses (d)
Figure 2  Farrowing rate (FR) (%) and number of piglets born alive per litter (BA) (a) and mummified fetuses/litter (%) (b) after the occurrence of abortion
Figure 3 Number of aborted sows from June to November 2008 in a commercial swine herd in Thailand.

Figure 4 Number of aborted gilts and sows from July to October 2008 by parity, Parity 0 = gilt (n=130)
Descriptive data on some reproductive traits of sows including farrowing rate (FR), number of piglets born alive per litter (BA) and the percentage of mummified fetuses per litter (%) are presented in Figures 2a and 2b. Reproductive performance of sows dramatically declined after the abortion epidemic. For instance, from March to October, the farrowing rate decreased from 86.7% to 65.6%, the number of piglets born alive per litter decreased from 10.4 to 8.6 piglets/litter, and the percentage of mummified fetuses increased from 3.3% to 9.3%.

Figure 3 showed number of aborted gilts and sows observed in the herd from June to November, 2008. A total number of 132 aborted gilts/sows were observed from July to October 2008. The number of aborted gilts and sows was dramatically reduced in November 2008. The numbers of aborted sows by parity and by period of gestation are shown in Figures 4 and 5, respectively. The occurrence of abortion was found both in gilts and sows (Figure 4). On average, the gilts/sows aborted at 50.4 days of gestation. Most of the gilts/sows aborted during the first trimester of gestation (25-48 days) (Figure 5).

During this period, Chlortetracycline (CTC) 600-800 ppm was medicatedly fed to the sows for 4 weeks (600 ppm for 2 weeks and 800 ppm for 2 weeks) in September. In October, sulfadimethoxine 500 ppm was used instead of CTC (Fig. 3).

**Discussion**

The causes of abortion were differentially diagnosed into several factors including management, seasons and diseases. Initially, the special attention was drawn to many management factors that may induce stress to the pregnant gilts and sows, such as feeding management, ambient temperature, stock persons and water supply. However, none of these factors was convincing after the history taking and farm visiting. Also, the summer-autumn abortion was taken into consideration, since the previous studies demonstrated that the high ambient temperature and/or high humidity during this period of the year in Thailand decreased the reproductive performances of gilts and sows (Tantasuparuk et al., 2000; Tummaruk et al., 2004; Suriyasomboon et al., 2006). However, based on the high incidence of abortion (4.5% in July to 15.2% in
October), the seasonal variation did not seem to play a major role. Thus, we then focused on the possible causative pathogens including viruses, bacteria and parasites. In this study, some of the common abortion-causing diseases in pig in Thailand were largely ruled out, for example, SF, AD, PRRS and PPV. The AD sero-prevalence of this swine herd was monitored annually and no incidence of AD was found in the past two years. Also, the gI of AD antibody titer from the tested sera revealed no AD infection among aborted gilts and sows. PRRS was also unlikely to be the cause due to the PRRS-positive herd history with relatively low titer for several years. For PPV, the high antibody titer of PPV was commonly observed in replacement gilts in Thailand in which 85-100% of replacement gilts had antibody titers against PPV ≥1:4096 (Tummaruk and Tatilertcharoen, 2008). Despite the routine practice of PPV vaccination, this disease is still endemic in many herds in Thailand. In this study, although the PPV titer of the sows and gilts was relatively high, it was not associated with the pattern of abortion. For instance, three sows aborted between 6-10 days before the sample collection had PPV titer below 1:512, while two gilts aborted one day before sample collection had a PPV titer of >1:4096. In addition, the response to the treatment of sulfa-trimetroprim suggested that the cause of abortion was unlikely to be viruses. For the bacteria, since this farm used the high doses of CTC (600-800 ppm) during the initial phase of abortion, *Streptococcus spp.* and *Leptospira spp.* were likely to be ruled out. Although the presence of high number of mice and rats could be associated with Leptospirosis, the serovar of *Leptospira spp.* detected from the sera was rather uncommon and did not usually cause high incidence of abortion (Suwanchareon and Kunavongkrit, 2000).

In addition to the above abortion-causing infectious agents, we demonstrated the occurrence of *T. gondii* antibody in aborted sows and gilts from this herd. Although the parasite is frequently observed worldwide (Dubey, 2004), little is known about the current prevalence of *T. gondii* among swine herds in Thailand. In the last two decades, a small number of reports on swine toxoplasmosis in Thailand were demonstrated in the sows and piglets by using latex agglutination test as well as presenting of tachyzoites in the tissues (Vayuchote et al., 1991; Tuntasuvan et al. 1990, 2000; Thiptara et al., 2006). In Argentina, the sero-prevalence of *T. gondii* among sows from 83 herds was 37.8% (87/230 sows) and the sero-prevalence of the parasites varied among provinces from 3.3% to 62.8% (Venturini et al., 2004). In addition, in a particular herd in Argentina, the incidence of the sero-positive pigs varied among age groups, e.g., 100% in sows, 40% in nursery piglets, 23.8% in growers and 20% in fatteners (Venturini et al., 2004). In Portugal, *T. gondii* sero-positive samples were found in 15.6% (52/333) of pigs prior to slaughter using modified agglutination test (MAT), and 15 isolates of *T. gondii* were obtained from 37 sero-positive pigs (47.5%) (de Sousa et al., 2006).

Since *T. gondii*-infected pigs are an important source of human infection (Dubey, 1986; 2004) the occurrence of *T. gondii* in the pig farm should be of concern for public health veterinarians. In the present study, a high prevalence of *T. gondii* sero-positive sows was observed. The reasons might be due to the presence of cats and rodents in the herd, feeding practices, and herd sanitation. In this herd, the feed was routinely left overnight in the feeder without covering in the gestation house. This type of food storage can increase the chance of infection, in which it is rather common among commercial swine herds in Thailand. As previously demonstrated, *T. gondii* sero-positive pigs were found in nearly all age groups from the herds with poor management and the presence of cats and rats (Venturini et al., 2004). These findings suggest that awareness should be raised regarding the occurrence of *T. gondii* in pig farms in Thailand. In particular, the control of infection could be improved by preventing the exposure to oocysts in cat feces and tissue cysts in dead carcasses.

In this study, the antibody against *T. gondii* of the aborted gilts/sows was tested by the latex agglutination method. This technique has already been used in a
number of studies and has been proven to have a rather high specificity and sensitivity (Dubey et al., 1997; Fun et al., 2001; Gamble et al., 2005; Jiang et al., 2008). However, some studies found that the latex agglutination test may have a relatively lower sensitivity compared to other serological tests, e.g. MAT, Enzyme linked immunosorbent assay (ELISA) and Sabin-Feldman dye test (DT) (Dubey et al., 1995; 1997). In general, the level of antibody titer against *T. gondii* using any method may vary according to strain of *T. gondii*, number of ingested oocysts, and the individual pig response (Dubey et al., 1997). For instance, a virulent strain of *T. gondii* (e.g., GT-1 strain) resulted in a titer level of 1:128 between 4 and 27 weeks after inoculation with approximately 1,000 oocysts in 2-5 month-old pigs using the latex agglutination test. Furthermore, the data were shown that 2.2% of *T. gondii*-infected pigs had no antibody in any of the serologic tests (Dubey et al., 1997).

In conclusion, *T. gondii* infection among aborted sows was observed in a commercial swine herd in Thailand. Of the 13 aborted gilts and sows that were randomly selected for blood sampling, we found 12 gilts and sows (92.3%) with positive titers 1:64-1:128. The occurrence of this disease not only decreases reproductive performance of sows and gilts but also suggests that awareness of the occurrence of *T. gondii* in pig farms in Thailand should be raised.

**Acknowledgement**

We would like to thank the herd owner and all the animal husbandry staffs for their cooperation, Novartis (Thailand) Co. Ltd. and Dr. Pawarit Chaiyota for technical assistance, Dr. Thanit Damrongwattanapokin and Dr. Duangjai Suwanchareon for their suggestions on Leptospirosis, and Dr. Sudarat Chaichomlert for suggestions concerning the latex agglutination test and Chula Unisearch, Chulalongkorn University, for proof reading the manuscript.

**References**


The Efficacy of *Escherichia coli* AroA-Live Vaccine in Broilers against Avian *E. coli* Serotype O78 Infection

Visut Rawiwet  Niwat Chansiripornchai*

Abstract

The efficacy of an *Escherichia coli* (*E. coli*) aroA-live vaccine in the prevention of colibacillosis in chickens following intratracheal challenge with a virulent strain of *E. coli* O78 was investigated. Thirty-six, one day old broiler chickens were divided into 3 groups of 12 each. Chickens in each group were randomly divided into 2 replicates. The chickens in group 1 that were not vaccinated and challenged served as a negative control. The chickens in group 2 that were not vaccinated but received *E. coli* serotype O78 served as a positive control. The chickens in group 3 were vaccinated by the oral route at 5 days of age with *E. coli* aroA-vaccine and challenged with *E. coli* serotype O78. All the chickens in groups 2 and 3 were challenged intratracheally at 4 weeks of age with 0.5 ml (1.2x10⁹ cfu/ml) per dose of *E. coli* O78. The chickens were monitored for 7 days after infection for feed conversion ratio (FCR), and the post-mortem pathology was assessed. The results revealed that the vaccine tends to prevent *E. coli* infection. The chickens in group 3 tended to show lower pathological findings including airsacculitis, pericarditis, perihepatitis, peritonitis and arthritis than the chickens in group 2 but the FCR was not different in each group (*p*>0.05).

**Keywords**: broilers, colibacillosis, *Escherichia coli* aroA-live vaccine, *E. coli* O78.

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Introduction

Colibacillosis, caused by *Escherichia coli* (*E. coli*), is a common systemic disease and has economic importance in poultry industry. *E. coli* infection occurs as an acute fatal septicemia or subacute pericarditis and airsacculitis, as well as perhepatitis, arthritis, and also cellulitis. Among bacterial infections, colibacillosis is very often the first cause of morbidity and mortality in poultry. A large number of *E. coli* are maintained in the poultry house environment through fecal contamination. Systemic infection occurs when a large number of pathogenic *E. coli* gain access to the blood stream via the respiratory tract or intestines. Bacteremia progresses to septicemia and death, or the infection extends to the serosal surfaces, the pericardium, the joints and the other organs. Surveys have been conducted in many parts of the world to determine the serotypes that are most frequently associated with diseases caused by *E. coli* variations according to geographic region but in most studies the common serotypes have been O1, O2, O35, and O78 (Sojika et al., 1965; Heller et al., 1977; Chansiripornchai and Sasipreeyajan, 2002). Treatment strategies include the control of predisposing infections or environmental factors and the early use of antibiotics. Unfortunately, a high frequency of resistance to tetracycline, oxytetracycline, chlortetracycline and doxycycline has occurred and more than 93% and 100% of *E. coli* isolates are resistant to erythromycin (Chansiripornchai et al., 1995). Furthermore, using of antibiotic drugs in the future will tend to be reduced and restricted in commercial farms so *E. coli* vaccines are an alternative way to prevent and control of *E. coli* infection.

*E. coli* vaccines include an inactivated vaccine, a live attenuated vaccine and a recombinant vaccine. Effective inactivated vaccines against various serotypes, including O2:K1 and O78:K80 have been produced (Deb and Harry, 1976; Deb and Harry, 1978; Cessi, 1979).
The inactivated vaccines provide protection against the homologous serogroups but little or no protection against the heterologous serogroups. Recently, a commercial live vaccine for chickens has been developed. The inventor has proclaimed that the \textit{E. coli} aroA-live vaccine can protect against the homologous and heterologous serogroups. However, the \textit{E. coli} aroA-live vaccine has never been proved to have the efficacy of vaccine protection for the virulent \textit{E. coli} serotype O78 isolated from Thailand.

The Poulvac® \textit{E. coli} vaccine contains an \textit{E. coli} strain that has been genetically-modified by the deletion of the aroA gene responsible for the biosynthesis of amino acids in the virulent \textit{E. coli} parent strain (The GMO is named aroA-PTA-5094). The aroA gene-deleted vaccine is capable of triggering a protective immunity in poultry against infection and disease from wild, virulent \textit{E. coli} bacteria found in the environment. However, because the aroA gene is deleted, the live vaccine bacterium is avirulent and unable to form a self-sustaining population since the vaccine strain has lost the capability to synthesize the amino acids necessary for its survival. The objective of the experiment was to prove the efficacy of the \textit{E. coli} aroA-live vaccine against \textit{E. coli} serotype O78 isolated from Thailand.

\textbf{Materials and methods}

\textbf{Chickens:} Thirty-six, unvaccinated broiler chickens (Arbor Acres) of mixed sex were obtained on the day of hatching from a commercial hatchery (Krungthai Farm, Thailand). The chickens were fed ad libitum before and during the experiments. At the onset of the experiments, no significant difference in average body weight between the experimental groups was found. The guidelines and legislative regulations on the use of animals for scientific purposes of Chulalongkorn University, Bangkok, Thailand were followed as is certified in permission No. 0831071.

\textbf{Bacterial strains:} The chickens were challenged with an \textit{E. coli} serotype O78 that had been originally isolated from the diseased air sacs of a chicken with a field case of colisepticemia (Chansiripornchai and Sasipreeyajan, 2002). In the experiment, 0.5 ml of the \textit{E. coli} suspension, containing $1.2 \times 10^9$ cfu/ml was used for intratracheal challenge. At the end of experiment, the livers of all the birds were collected for \textit{E. coli} identification.

\textbf{Vaccination and Experimental designs:} \textit{E. coli} aroA-vaccine (Poulvac \textit{E. coli}, Fort Dodge Animal Health, Iowa, USA) was orally administered to the chickens according to the manufacture’s recommendation. The \textit{E. coli} vaccine dosages were calculated according to a titer of $5.0 \times 10^6$ cfu per dose. Thirty-six, unvaccinated broiler chickens of mixed sex were divided into 3 groups of 12 each. Chickens in each group were randomly divided into 2 replicates. Group 1 that was not vaccinated and challenged served as a negative control group. Group 2 that was not vaccinated but was challenged served as a positive control group. Group 3 was orally vaccinated at 5 days of age with \textit{E. coli} aroA-vaccine and received \textit{E. coli} serotype O78. All the chickens in groups 2 and 3 were intratracheally challenged at 28 days of age with 0.5 ml ($1.2 \times 10^9$ cfu/ml) per dose of \textit{E. coli} O78. At 7 days post challenge, the number of dead birds was noted and all the surviving birds were necropsied and examined for the presence of grossly visible lesions of colibacillosis.

\textbf{Efficacy criteria and definitions:} Mortality was defined as the number of chickens that were killed or that had died before the end of the trial. Morbidity was defined as the number of birds with lesions in either airsac, pericardium or perihepatic.

The airsac, pericardial and perihepatic lesions of colisepticemia in each bird were scored. The airsac lesions of colisepticemia were scored according to Kleven et al. (1972) as follows: 0: no lesions, 1: cloudiness of air sacs, 2: air sac membranes are thickened, 3: “meaty” appearance of membranes, with large accumulations of a cheesy exudate confined to one air sac, 4: lesions with the same score as score 3 but with lesions in two or more air sacs. The pericardial lesions of colisepticemia were scored according to
Charleston et al. (1997) as follows: 0: no lesions, 1: excessive clear or cloudy fluid in the pericardium, 2: extensive fibrination in the pericardial cavity. The perihepatic lesions of colisepticemia were scored according to Charleston et al. (1997) as follows: 0: no visible lesions, 1: definite fibrination on the surface of the liver, 2: extensive fibrination, adhesions, liver swelling and necrosis.

Chickens with severe lesions were characterized as having an air sac lesion score of 4 and pericarditis and perihepatitis scores of either 1 or 2. The average body weight of the birds in each group was measured at 1 day, 28 days and 35 days of age. A feed conversion ratio (FCR) was calculated for each group by taking the total amount of feed consumed by each group between days 1-35 and dividing it by the increase in mass of the chickens over the same time period.

Statistical analysis: ANOVA and Duncan multiple range tests were used for the statistical comparison of the body weight. The mortality, morbidity and the lesion scores were analysed by Chi-square and Mann-Whitney U test, respectively. SPSS for Windows was used for statistical analysis.

**Results and Discussion**

Pathological findings and production parameters:

One chicken in each group was culling before vaccination due to drowning. The pathological findings and production parameters measured for vaccinated or control groups are summarized in Table 1. Two birds died in the positive control and the vaccinated groups at one and two days post challenge with *E. coli* O78, respectively. The birds were found to have acute, severe septicemia and *E. coli* could be isolated from the livers at postmortem examination.

The mortality and morbidity rate of the birds vaccinated with *E. coli* aroA-live vaccine was no different from the positive control group (*p*>0.05). For the pathological findings, the average lesion scores of airsacs and the number of birds with arthritis in the group vaccinated with *E. coli* aroA-live vaccine were statistically significantly less than those of the positive control group (*p*<0.05). There was no difference in the FCR among the 3 groups (*p*>0.05).

Bacteriology: All the isolates of birds in groups 2 and 3 were *E. coli* serotype O78 which was the same serotype as the *E. coli* challenge strain. The birds in group 2 with no vaccination had a significantly higher number of the *E. coli* isolates than those birds in group 3 vaccinated with one dose at 5 days old (*p*<0.05) (table 1).

In the positive control group, broilers that were challenged with *E. coli* serotype O78 revealed 9% mortality and 91% morbidity. Normally, the mortality rate in field cases caused by *E. coli* infection is 0.25% in the primary and increases to 1% after being infected for 5 days and can increase up to 10% (Shane, 1981; Wrey et al., 1996). Fan et al. (2004) showed a mortality of 28.1% for chickens that were intratracheally challenged with 1.0 x 10^9 cfu *E. coli* O78 at 6 weeks of age. In contradiction of Rosenberger et al. (1985), 0% mortality was found in the one day old chickens after being intratracheally challenged with the concentration of less than 10^4 cfu of *E. coli*, but the mortality increased to 60% when the challenge dose was increased to 10^7 cfu. At 15 days of infection, the chickens apparently developed an age-associated resistance to *E. coli* introduced intratracheally. In our model, mortality could be found after the chickens had been challenged with 0.6 x 10^9 cfu/ml *E. coli* O78 at 28 days old. Thus, the birds vaccinated with *E. coli* aroA-live vaccine could have reduced morbidity but not a reduced mortality rate. These results do not all agree with Fan et al. (2004), showing that birds vaccinated with *E. coli* aroA-live vaccine can have a reduce morbidity and mortality rate. In this experiment, there was no difference in mortality rate between the vaccinated and the positive control group but the chickens in the vaccinated group died later than the chickens in the positive control group. In the pathological findings, lesion scores of airsacs, pericardium,
<table>
<thead>
<tr>
<th>Group</th>
<th>Morbidity</th>
<th>Mortality</th>
<th>No. of birds with gross lesions</th>
<th>Mean gross lesion score ± SD and No. of birds with gross lesions</th>
<th>% birds positive for E. coli</th>
<th>No. of bird FCR day</th>
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<tr>
<td></td>
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<td></td>
<td>Peritonitis</td>
<td>Arthritis Airsacculitis&lt;sup&gt;y&lt;/sup&gt; Pericarditis&lt;sup&gt;z&lt;/sup&gt; Perihepatitis&lt;sup&gt;z&lt;/sup&gt;</td>
<td>lesions&lt;sup&gt;*&lt;/sup&gt;</td>
<td>1-35</td>
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<tr>
<td>1 (n=11)</td>
<td>0/11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/11&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>2 (n=11)</td>
<td>10/11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1/11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2/11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.82±1.33&lt;sup&gt;b&lt;/sup&gt; (10/11&lt;sup&gt;c&lt;/sup&gt;) (8/11&lt;sup&gt;b&lt;/sup&gt;) (1/11&lt;sup&gt;a&lt;/sup&gt;)</td>
<td>0.91±0.70&lt;sup&gt;b&lt;/sup&gt; (1/11&lt;sup&gt;a&lt;/sup&gt;)</td>
<td>5/11&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>1/11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.82±1.40&lt;sup&gt;a&lt;/sup&gt; (4/11&lt;sup&gt;b&lt;/sup&gt;) (6/11&lt;sup&gt;b&lt;/sup&gt;) (0/11&lt;sup&gt;a&lt;/sup&gt;)</td>
<td>0.55±0.52&lt;sup&gt;b&lt;/sup&gt; (6/11&lt;sup&gt;b&lt;/sup&gt;)</td>
<td>1/11&lt;sup&gt;a&lt;/sup&gt;</td>
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</table>

<sup>*</sup>Birds with severe lesions were characterized as having an air sac lesion score of 4 and pericarditis and perihepatitis scores of either 1 or 2.
<sup>x</sup>Air sac lesions were scored on a scale of from 0 to 4.
<sup>y</sup>Pericardium lesions were scored on a scale of from 0 to 2.
<sup>z</sup>Perihepatic lesions were scored on a scale of from 0 to 2.
<sup>a,b</sup>The superscripts that differ in each column have significantly different at confidential 95% (p<0.5).

Group 1: negative control; Group 2: positive control; Group 3: vaccination and challenge.
perihepatic and the number of birds with peritonitis and arthritis were investigated. The birds in the positive control group had a significantly higher airsac lesion score and also a higher number of birds with arthritis than the birds in the vaccinated group \( (p<0.05) \). Compared to the vaccination and the positive control group, chickens in the vaccinated group tended to show lower morbidity and fewer pericarditis- and perihepatitis lesion scores than the chickens in the positive control group. In conclusion, the \textit{E. coli} \textit{aroA}-live vaccine tends to reduce the pathological lesions of the chickens challenged with \textit{E. coli} serotype O78 isolated in Thailand.

**Acknowledgements**

Thanks are expressed for Ratchadaphiseksomphot Endowment Fund, Chulalongkorn University for supporting this work.

**References**


Effects of Permethrin at a Prophylactic Dose for Ectoparasite Infection on Cholinesterase Activity in Dogs

Piyarat Chansiripornchai* Niwat Chansiripornchai

Abstract

Effects of permethrin at a prophylactic dose for ectoparasite infection on cholinesterase (ChE) activity was evaluated. Eight healthy dogs were exposed to a spot-on formulation of permethrin on day 0. The results of serum ChE activity in all of the dogs which was measured at 24 h after exposure to permethrin significantly decreased when compared to those before exposure. However, the serum ChE activity in all the dogs which was measured at 36 and 96 h after exposure to permethrin showed no significant difference as compared to those before exposure. There were no clinical signs in all dogs throughout the experiment. The results suggest that using spot-on formulation of permethrin at a prophylactic dose for ectoparasite infection is safe in dogs.

Keywords: cholinesterase, dog, permethrin, toxicity

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**Introduction**

Permethrin is a pyrethroid insecticide used in both agricultural and domestic products (Sutton et al., 2007). Permethrin is a type I pyrethroid which has a low toxicity compared with type II pyrethroid such as cypermethrin (Rao and Rao, 1995). Permethrin acts on voltage-dependent sodium channels by extending the channel opening causing an increased sodium current; consequently, depolarization is prolonged leading to a repetitive firing of the nerve (Ray, 1991; Sutton et al., 2007). Moreover, permethrin inhibits ChE by interaction at the hydrophobic aromatic surface region of the enzyme, thus reducing in ChE activity (Rao and Rao, 1995).

Permethrin is used in numerous formulations for the control of insect on pets such as ticks on animals and in environment. In Thailand, spot-on formulation of permethrin for dogs is available at a concentration of 65% (w/v). Since cats are sensitive to permethrin either by direct contact or secondary exposure through contact with other pets treated with permethrin (Merola and Dunayer, 2006) the product is contraindicated in this species.

In general, permethrin is considered to be of low toxicity to dogs when used as directed by the manufacturer (Richardson, 1999). However, there have been many reports on the toxicity of pyrethroid insecticides in dogs (Bates, 2000; Martin and Campbell, 2000). The lethal toxicity of pyrethroid in animals has been published but the sublethal toxicity of the agent has not been studied, especially, the effect of permethrin at the prophylactic dose that is used in small animal practices. It is accepted that exposure to pyrethroid insecticides such as permethrin can decrease cholinesterase enzyme (ChE) activity which affects the nervous system function (Balint et al., 1995; Hakbrook et al., 1992; Rao and Rao, 1995). Measurement of ChE activity appears to have been useful in monitoring the sublethal toxicity of insecticides and also in the safety evaluation of the drug’s use prior to clinical signs are being detected (Chansiripornchai et al., 2008).

This study was performed to evaluate the effect of permethrin spot-on formulation at a prophylactic dose for ectoparasite infection on ChE activity in dogs.
Materials and Method

Eight healthy dogs with normal hematological and biochemical profiles from a private animal hospital were used as experimental animals. On day 0, blood samples were taken from all the dogs for the measurement of alanine aminotransferase (ALT) and the creatinine for liver and kidney function test, respectively. ChE activity was also measured. After taking the blood, a spot-on formulation of permethrin (Protical®, Schering Plough, Germany) at the recommended dose of 65% (w/v) was administered to the back of all the dogs and they were kept in a conventional area. At 24, 36 and 96 h after exposure to permethrin, blood samples were taken from all dogs for measurement of ChE, ALT and creatinine. The ALT and creatinine levels were analyzed using an automatic analyzer (FujiDri-Chem 3500i, Fuji, Japan). The ChE activity was measured as described by Ellman et al. (1961) using colorimetric determination by spectrophotometer (UV-160a), Shimadzu, Japan). Data was analyzed using the repeated measure ANOVA (p<0.05).

Results and Discussion

Pyrethroids are recognized as the fourth major class of insecticides and they interfere with the function of the nervous system like other major classes of insecticides such as organochlorines, organophosphates and carbamates (Elliot, 1997). The widespread use of pyrethrins and pyrethroids has increased in animals and humans and has led to an interest in their toxicoses. Dermal exposure of permethrin by application to the skin and hair coat is the most frequent route that leads to intoxication, particularly in cats (Anadon et al., 2009). Although pyrethrins and pyrethroids are generally regarded as safe in dogs, their sublethal toxicity should be evaluated for toxicological data. The determination of serum ChE activity is used for the evaluation of intoxication by organophosphate, carbamate and pyrethroid insecticides which are ChE inhibitors (Balint et. al., 1995; Halbrook et. al., 1992; Rao and Rao, 1995). The colorimetric method for the measurement of ChE activity as described by Ellman et al. (1961) using thiocholine ester as a substrate and 5, 5'-dithiobis-(2- nitrobenzoic acid) as a coloring agent offers good accuracy and is widely used (Okabe et al., 1997).

In the present study, the effect of 65% (w/v) permethrin spot-on formulation at a prophylactic dose for ectoparasite infection in dogs was evaluated by the measurement of ChE activity. As a result, serum ChE activity in all the dogs significantly decreased within 24 h after exposure to permethrin (Figure 1). This result may concur with the study determined by Ansari et al. (1990) which found that using cypermethrin, a pyrethroid insecticide, at a prophylactic dose for ectoparasite infection in cattle can decrease ChE activity on day 1 after exposure and can increase to the normal level within 7 days after exposure. However, the serum ChE activity in the dogs at 36 hr after exposure increased and showed no significant difference when compared with that before exposure. Moreover, there were no clinical signs in any dogs throughout the experiment and the ALT and creatinine levels were also within normal range (Table 1).

On the other hand, a previous report of using a combination spot-on formulation of permethrin and imidacloprid (Advantix®, Bayer, Germany) at a prophylactic dose for ectoparasite infection in dogs indicates that the 60% (w/v) of permethrin in this product did not have any effect on serum ChE activity (Chansiripornchai et al., 2008) but using 65% (w/v) of permethrin in our present study decreased the ChE activity within 24 h after exposure. This result suggests that using a higher concentration of permethrin may effect ChE activity and may cause clinical signs in the animal. Since 65% (w/v) permethrin spot-on formulation is the highest concentration of this product in Thailand, therefore it is safe to use the product for dogs.
Figure 1. Serum cholinesterase activity (micromole of substrate hydrolyzed/min/ml) in the dogs after exposure to permethrin (n = 8)

Table 1. The ALT and creatinine levels in dogs on day 0, 1 and 4 after exposure to permethrin

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>D0</th>
<th>D1</th>
<th>D4</th>
<th>D0</th>
<th>D1</th>
<th>D4</th>
</tr>
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<tr>
<td>1</td>
<td>31</td>
<td>30</td>
<td>29</td>
<td>1.1</td>
<td>0.9</td>
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<td>2</td>
<td>25</td>
<td>23</td>
<td>26</td>
<td>0.7</td>
<td>0.7</td>
<td>0.9</td>
</tr>
<tr>
<td>3</td>
<td>31</td>
<td>31</td>
<td>28</td>
<td>0.8</td>
<td>0.9</td>
<td>0.8</td>
</tr>
<tr>
<td>4</td>
<td>38</td>
<td>41</td>
<td>37</td>
<td>0.8</td>
<td>0.9</td>
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</tr>
<tr>
<td>5</td>
<td>45</td>
<td>49</td>
<td>53</td>
<td>1.0</td>
<td>0.8</td>
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<tr>
<td>6</td>
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<td>21</td>
<td>0.5</td>
<td>0.6</td>
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<tr>
<td>7</td>
<td>32</td>
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<td>31</td>
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<td>0.5</td>
</tr>
<tr>
<td>8</td>
<td>28</td>
<td>29</td>
<td>26</td>
<td>1.1</td>
<td>1.2</td>
<td>1.2</td>
</tr>
</tbody>
</table>

*Normal level 8.2-57.3 (IU/l) (Fraser et al., 1991), **Normal level 0.5-1.6 (mg/dl) (Fraser et al., 1991)
Acknowledgments
We wish to thank Mr. Wiritpol Chanwaowam, Miss Sukolapa Chiarasumran and Miss Boonyarat Temeecharoentaworn for laboratory assistance.

References
These lead II ECG strips were recorded from a 13 years old, female, spayed, English Cocker Spaniel, weighing 15.2 kg. The first and the second strips were recorded with a paper speed of 25 and 50 mm/second, respectively. The dog had history of syncope, exercise intolerance and anorexia. Clinical examination revealed pink mucous membrane and normal hydration status. Auscultation of the chest revealed bradycardia and increased lung sound. Complete blood count and serum chemistry profiles were within normal limits.

Please answer before turning to the next page.
Interpretation

Sinus rhythm with third degree atrioventricular block or complete atrioventricular dissociation

The impulses originated from sinus cannot pass through ventricle at all and the blockage is at the AV junction. The presence of multiple P-waves (dark arrows) without following normal QRS complexes is the hallmark of AV block. No PR interval was found. The atrial rate in this case is approximately 180 beats/minute. Since the impulses do not reach the ventricular muscle, the ectopic pacemaker originated below the AV node is emerged. The shape of ventricular action potentials is bizarre (big open arrows) and the duration is prolonged due to abnormal pathway transmission besides the His bundle. The ventricular escape rhythm was paced at a rate of 45 beats/minute, which is much slower than the sinus rate. Tissue perfusion is disrupted by low ventricular rate resulting in low cardiac output. The animals usually show signs of syncope or severe exercise intolerance. The complete block of AV transmission suggests that the pathologic lesion such as degeneration or fibrosis occurs at the area of AV node. However, the location of block may be His bundle or bundle branch fascicles which usually do not respond to cardiac drugs. Decreased parasympathetic tone by atropine or increased sympathetic tone by sympathomimetic drugs such as dobutamine, isoproterenol, theophylline, aminophylline may change the ventricular response rate in some cases. Giving atropine in this case at the dose of 0.04 mg/kg once daily makes the ventricular rate faster but the complete block is not disappeared. The dog shows better clinical signs when the ventricular rate was speeded up to 55 beats/minute after atropine treatment without other complications. However, the atrial rate was also elevated to 220 beats/minute. The choice of treatment in complete AV dissociation is pacemaker implantation.
Ophthalmology Snapshot

Nalinee Tuntivanich

History

A 10 yr-old female mixed breed dog was presented at the Small Animal Teaching Hospital, Chulalongkorn University with a proliferative, bright red mass at the outer surface of the left nictitating membrane (Figure 1A). The mass had been presented for several months and slowly enlarged lately. Neither ocular pain nor hemorrhage was observed. Fluorescein staining test revealed no stromal corneal ulcer. A combination of antibiotic and corticosteroid ointment was topically administered twice daily for three consecutive days prior to surgery. The mass appeared less proliferative at day of surgery (Figure 1B).

Figure 1. Photograph of the left eye at (A) day of ophthalmic examination revealing a proliferative, bright red mass at the nictitating membrane, and (B) day of surgery (the dog was under general anesthesia).

Questions

Give your tentative diagnosis, treatment of choice and prognosis?

(For better quality, figures can be viewed in the TJVM website.)

Please turn to the next page for answers .....
Comments

Tumor of vascular endothelial origin is common in dogs. Hemangioma accounts for approximately 2% of conjunctival tumor in canine. Average age of onset is 8.6 years. Although there is no gender predilection, female dogs may have higher risk since level of estrogen was reported to be elevated in the blood of hemangioma patients. The leading edge of the nictitating membrane is the most common site of involvement. Dogs that spend great amount of time outdoors increase risk to develop this type of tumor. There is a report indicating that there is an expression of proteins potentially altered by UV exposure in canine hemangioma as well as hemangiosarcoma. Diagnosis can be made by typical clinical appearance; however biopsy is required for definitive diagnosis. Surgical excision (with/without additional treatment) may be curative, though recurrence is still possible.

Additional information

Treatment of this case: the tumor was surgically removed. Electrotherapy was applied afterward at wider region around the surgical site.

Final histopathological diagnosis: hemangioma

No evidence of recurrence has been noted until the last ophthalmic examination at 5 months after treatment.

References


Ultrasound Diagnosis

Phiwipha Kamonrat

History

A ten-year-old, spayed female, Labrador Retriever dog was referred to the Chulalongkorn University, Small Animal, Veterinary Teaching Hospital for investigation of progressive abdominal enlargement. The dog had recently been treated for atopic dermatitis and hypothyroidism. Clinical examination revealed pale mucous membranes and a tense abdomen on palpation of the left mid abdomen. The dog had anemia, thrombocytopenia and increased serum alanine aminotransferase (1,000 units) and alkaline phosphatase (620 units). The blood urea nitrogen (13 mg/dl) and creatinine (0.6 mg/dl) were within normal limits. On diagnostic radiography, there was a well-defined, circular, approximately 15x19 cm, lesion with soft tissue opacity on the left mid-ventral quadrant of the abdominal cavity. Most of bowel loops were displaced to the right abdomen. Ultrasonography of the mass lesion and the entire abdomen was performed.

Ultrasonographic Findings

Real-time, ultrasonographic images were obtained using an 8 MHz microconvex, phased array transducer with the dog in dorsal recumbency. A mid-ventral soft-tissue mass seen on radiographs was ultrasonographically well-defined, heteroechoic, solid, 14 by 18 cm in diameter and contained multicystic anechoic areas with irregular margins (Figure 1A and 2A). A border of the mass was smooth except in cranial portion of the mass which was irregular and involved the cranial pole of the left kidney (Figure 1B and 2B). The larger part of the left kidney was still normal in echotexture, with a good corticomedullary definition. There were multifocal, hypoechoic, circumscribed nodules, 0.2-2.2 cm in diameter diffused in the splenic tail. Sonography of other abdominal organs including the right kidney, urinary bladder and abdominal lymph nodes appeared normal in echotexture.
Figure 1 An Ultrasonographic image of the left mid-ventral abdominal mass, of a ten-year-old, spayed female, Labrador Retriever dog in dorsal recumbency. This mass was well-defined and heteroechoic with diffuse multicystic anechoic areas (A). A cranial portion of the mass was lobulated and involved the cranioventral part of the left kidney.

Figure 2 Schematics of the relative positions of the left renal mass scanned in figure 1. M-mass; A-anechoic areas of the mass; K-renal parenchyma.
This solid mass was confirmed to be a left renal mass at surgery and histopathologically diagnosed as renal hemangioma.

**Diagnosis**

Ultrasonographic diagnosis——A renal solid mass (hemangioma).

**Comments**

Ultrasonography is the method of choice in the detection and differentiation of solid or cystic masses in the abdomen. It is less specific for diffuse renal disease than focal or multifocal renal abnormalities. It also has limitations in differentiating benign from malignant masses. In humans, tumor vascular signals detected with Doppler ultrasonography is helpful in discriminating between benign and malignant renal masses (Ramos et al., 1988). There is a large variation in the ultrasonographic appearance of canine renal neoplasms (Konde et al., 1985). The most common pattern is a complex mass that contains a variable mixture of anechoic, hypoechoic and hyperechoic components.

In dogs, the most common benign renal tumor is hemangioma (Eddlestone et al., 1999). It may have significantly variable ultrasonographic features, which may mimic hemangiosarcoma that appears as a focal hyperechoic or complex mass. For a very large mass as found in this dog, it is hard to specify whether the mass originates from the kidney or it has displaced the kidney from its normal location. Therefore, the accurate diagnosis of the renal tumor origin or cell type and whether the tumor was benign or malignant must be confirmed by cytologic or histopathologic examination of an ultrasound-guided biopsy/aspiration, endoscopic or surgical specimen.

**References**


What is Your Diagnosis

Pranee Tuntivanich
Suwicha Chuthatep

Signalment:
A 6-year-old male Golden retriever.

History:
The dog had had megaesophagus for a year and had continuing medical treatment. Severe regurgitation and vomiting had been presented for the past 5 days before the dog was presented at the hospital.

Clinical Examination:
The dog showed mild abdominal pain. There was no evidence of abnormal mass via abdominal palpation. Hematologically, anemia and mild increase of liver profile could be detected. Blood electrolyte profile showed alkalosis.

Radiographic Examination:
In addition to plain thoracic radiographs, positive contrast esophagography (barium sulfate swallow) were performed to evaluate esophageal abnormalities.

Figure 1. Right lateral (A, B) and ventrodorsal (C) thoracic plain radiographs.
Figure 2. Right lateral thoracic radiograph taken immediately after barium sulfate swallow.

Give your diagnosis and turn to the next page.
Radiographic findings

Plain lateral radiograph of the cranial thoracic portion (Figure 1A) revealed dilation of an esophagus resulting ventral displacement of the thoracic trachea and cranial heart base. A large soft tissue-like mass in the mid-caudodorsal thoracic cavity was presented in the plain lateral and ventrodorsal views of the caudal thoracic radiographs (Figures 1B and 1C). Absence of barium sulfate in the stomach in the contrast esophagogram (Figure 2) indicate an obstruction in the caudal esophagus. Small amount of barium sulfate could however be detected in the proximal part of caudal bronchi (Figures 2 and 3).

Radiographic diagnosis

Gastroesophageal intussusception (GEI)

Figure 3. Majority of barium sulfate was accumulated in front of caudal esophagus caused by GEI (large arrows). The radiopaque lines (small arrows) presented in the proximal portion of the bronchial walls was from barium sulfate aspiration during esophagography.

Discussion

Gastroesophageal intussusceptions (GEI) is a retrograde invagination of the stomach into the caudal part of the esophagus that was often reported in young German Shepherd dogs. Dogs with acute clinical signs are usually presented with severe respiratory compromise, resulting from compression of caudal lung lobes, while those with chronic signs presented with chronic regurgitation and vomiting.

GEI can be diagnosed on plain radiographs which reveal an enlargement of esophagus seen as a large oval-shaped filling defect in thoracic radiographs together with a cranial displacement of the stomach in abdominal radiographs. Contrast esophagography is a useful diagnostic technique but contrast aspiration can easily occurs due to swallowing difficulty. Esophagoscopy can also be used to differentiate GEI from esophageal foreign body and soft tissue mass in caudal esophagus.

References


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“Era of Modern Diagnostic Pathology”

November 19-20, 2009 Bangkok, Thailand

Organized by

The Asian Society of Veterinary Pathologists (ASVP)
The Veterinary Pathology Society of Thailand (VPST)
The Thai Association of Veterinary Laboratory Diagnosticians (TAVLD)
The Veterinary Oncology Center, Innovation Center for Veterinary Teaching and Services in Companion Animals
The Faculty of Veterinary Science, Chulalongkorn University, Thailand

Joint with

The Zoological Park Organization under the Royal Patronage of His Majesty the King
Chulalongkorn University Emerging and Re-emerging Infectious Diseases in Animals Center (CUEIDA)
Asian Foundation for the Advancement of Veterinary and Animal Sciences (AFAVAS)
CL Davis Foundation for the Advancement of Veterinary and Comparative Pathology
The Federation of Asian Veterinary Associations (FAVA)
Asian Society Wildlife Pathology and Parasitology (ASWPP)
The National Taiwan University
Organizing Committee

Proceeding of the 4th Asian Society of Veterinary Pathologists (ASVP)
Conference and Annual Meeting of The Thai Association of Veterinary Laboratory
Diagnosticians (TAVLD)

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                Taweewan Tansathit
                Tanongsak Mamom
                Angkana Somanataweechai
“The 4th Asian Society of Veterinary Pathologists Conference”

The 4th ASVP 2009

19-20 November 2009

Chulalongkorn University, Bangkok, Thailand

Opening Ceremony

Thursday 19th November 2009

Institute Building III Auditorium, Chulalongkorn University

Opening address

By Prof. Dr. Pirom Kamonrattanakul

President of Chulalongkorn University

Honorary President of the Asian Society of Veterinary Pathologists and the Chairman of the Organizing Committee of the 4th Asian Society of Veterinary Pathologists Conference, Dean of the Faculty of the Veterinary Science, Chulalongkorn University, the Faculty member committee, the President of the Thai Association of the Veterinary Laboratory Diagnostician, Head of the Innovation Center for Veterinary Teaching and Services in Companion Animal, Deputy Director of the Zoological Park Organization under the Royal Patronage under His Majesty the King, President of Asian Foundation for the Advancement of Veterinary and Animal Science, CL Davis Foundation for the Advancement of the Veterinary and Comparative Pathology, President of the Federation of Asian Veterinary Associations, President of the Asian Society Wildlife Pathology and Parasitology, Representative of National Taiwan University, Guest speakers, the participants, distinguished guests ladies and gentlemen,

It is a great honor for me to give the opening address of the 4th Asian Society of Veterinary Pathologists Conference which is held at Chulalongkorn University, Bangkok, Thailand on 19-20 November 2009. On behalf of Chulalongkorn University, I would like to welcome all the participants to the conference. Under the theme of “Era of the Modern Diagnostic Pathology”, emphasizes the progress of the diagnostic pathology with discovery of emerging diseases concerns human, animals and environment, I hope all of you will get benefits from the meeting by sharing experiences and learning from persons who share the same academic interests and eventually be able to implement the Pathological knowledges for prevention and control of animals and also wildlife health. Please continue our good friendship and collaboration in this field to promote an active and fruitful 4th ASVP 2009.

I would like to take this opportunity to thank all of the organizers for their supports, Faculty of Veterinary Science, Chulalongkorn University, as the host. Most of all, I wish to thank the Organizing Committee, the Thai Association of the Veterinary Laboratory Diagnostician, the Veterinary Oncology Center in the Innovation Center for Veterinary Teaching and Service on Companion Animal, the Zoological Park Organization under the Royal Patronage of His Majesty the King, all the International organizations, private sectors, and delegates who have contributed to organize and promote the 4th ASVP 2009. Let us open the conference and wish that we are going to have a successfully cooperation and friendship.

Thank you
“The 4th Asian Society of Veterinary Pathologists Conference”
The 4th ASVP 2009
19-20 November 2009
Chulalongkorn University, Bangkok, Thailand
Opening Ceremony
Thursday 19th November 2009
Institute Building III Auditorium, Chulalongkorn University

Report

by Assoc. Prof. Dr. Achariya Sailasuta
President of the Asian Society of the Veterinary Pathologists and Chairman of the Organizing Committee of the 4th ASVP 2009

The honorary President of Chulalongkorn University, Dean of the Faculty of the Veterinary Science, the Faculty member committee, President of the Thai Association of the Veterinary Laboratory Diagnostician, Head of the Innovation Center for Veterinary Teaching and Service in Companion Animal, Deputy Director General the Zoological Park Organization under the Royal Patronage of H.M. the King, the international committee, President of Asian Foundation for the Advancement of Veterinary and Animal Science, CL Davis Foundation for the Advancement of the Veterinary and Comparative Pathology, President of the Federation of Asian Veterinary Associations, President of the Asian Society Wildlife Pathology and Parasitology, Representative of National Taiwan University, Guest speakers, the participants, distinguished guests ladies and gentlemen,

The Faculty of Veterinary Science, Chulalongkorn University is honored by the Asian Society of the Veterinary Pathologists to host of the 4th Asian Society of the Veterinary Pathologists Conference under the theme “Era of Modern Diagnostic Pathology” with the collaboration of the Thai Association of Veterinary Laboratory Diagnostician, the Veterinary Pathology Society of Thailand, the Veterinary Oncology Center in the Innovation Center for Veterinary Teaching and Services in Companion Animal, the Zoological Park Organization under the Royal Patronage of His Majesty the King, Asian Foundation for the Advancement of Veterinary and Animal Science, CL Davis Foundation for the Advancement of the Veterinary and Comparative Pathology, The Federation of Asian Veterinary Associations, Asian Society Wildlife Pathology and Parasitology and National Taiwan University. This is the first time of the conference in Thailand. The conference is held at the Institute Building III Auditorium, Chulalongkorn University, during 19-20 November 2009. It includes the renowned 11 guest lectures, 61 poster presentations and 9 pathology slide discussion. The conference bring together approximately 180 participants from Asian and other regions of the world. All of whom will have a chance to discuss and exchange ideas or experiences with mutual interest in diagnostic pathology including oncology, zoo and wildlife pathology and emerging diseases on veterinary public health concerns. The organizing committee is very grateful to the sponsorships from private sectors.

I would like to take this opportunity to invite the President of Chulalongkorn University, Prof. Dr. Pirom Kamolrattanakul to give an opening address for the 4th Asian Society of the Veterinary Pathologists Conference.
Program of the 4th Asian Society of Veterinary Pathologists (ASVP)  
Conference and Annual Meeting of The Thai Association of Veterinary Laboratory Diagnosticians (TAVLD)

Thursday 19 November  2009

08:00-08:45 AM  Welcome Talk & Meeting and Registration
08:45-8:50 AM  The Opening Ceremony:
Prof. Dr. Pirom Kamolrattanakul
President of Chulalongkorn University

09.00-12.00 AM  Plenary session: Emerging and Re-emerging Disease in Animals: Epidemiology, Diagnosis, Control and Prevention
09:00-09:30 AM  A Visit from an Old Friend, Swine Influenza
Prof. Dr. Roongroje Thanawongnuwech*,
CUEIDA, Chulalongkorn University, Thailand

09:30-10:00 AM  Avian Influenza-Current and Perspective
Assoc. Prof. Dr. Alongkorn Amornsin,
CUEIDA, Chulalongkorn University, Thailand

10:00-10:30 AM  Avian Influenza in Animals
Assoc. Prof. Dr. Taweesak Songserm,
Kasetsart University, Thailand

10.30-10.45 AM  Morning Coffee Break

10:45-11.30 AM  Suppression of Rabies Virus Propagation in Mice Brain by Intracerebral Immunization of Inactivated Virus
Prof. Dr. Takashi Umemura,
President of the Japanese Society of Veterinary Pathologists

11.30-12.10 AM  Mammalian Models for Studies of Transmission of Highly Pathogenic Avian Influenza A (H5N1) Viruses with Meat from Infected Poultry
Dr. Yong Kuk Kwon
The National Veterinary Science and Quarantine Service (NURQS), Republic of Korea

12:10-01:30 PM  Lunch

01:30-02.45 PM  Necropsy Show and Tell
Prof. Dr. Nam Yong Park,
The CL Davis Foundation for the Advancement of Veterinary and Comparative Pathology, Republic of Korea

02.45-03.00 PM  Afternoon Break and Poster Session
03.00-04:30 PM  Descriptive Gross and Microscopic Veterinary Pathology, Necropsy, Biopsy and Surgical Pathology  
Prof. Dr. Paul. C. Stromberg,  
The CL Davis Foundation for the Advancement of Veterinary and Comparative Pathology, USA

04:30-05.15 PM  Pathology of Hepadna Virus Infection in Humans and Animals  
Prof . Dr. Kenji Abe,  
National Institute of Infectious Diseases, Japan

05.15-06.00 PM  Clinical Trials of Adipose Derived Stem Cells and Muscle Derived Stem Cells  
Prof. Dr. Kyu-Shik Jeong,  
Kyungpook National University, Republic of Korea

04.30-06.00 PM  Meeting of The Asian Society of Veterinary Pathologist (ASVP)  
Meeting of The Veterinary Pathology Society of Thailand (VPST)  
Meeting of The Thai Association of Veterinary Laboratory Diagnosticians

06:30-08:30 PM  Welcome Reception

Friday 20 November 2009

08:30-10:00 AM  Diagnostic Cytology in Veterinary Clinical Medicine  
Prof. Dr. Hiroki Sakai,  
Gifu University, Japan

10:00-10:30 AM  Morning coffee/Poster Session

10:30-12:00 AM  An Oncological Histopathological and Cytological Diagnosis  
Prof. Dr. Donald Meuten,  
The CL Davis Foundation for the Advancement of Veterinary and Comparative Pathology, USA  
President of American College of Veterinary Pathology (ACVP)  
Prof. Dr. Mary Anna Thrall,  
The CL Davis Foundation for the Advancement of Veterinary and Comparative Pathology, USA

12:00-01:30 PM  Lunch

01:30-04.30 PM  Slide Discussion  
Poster Session

04.30-05.00 PM  Closing Ceremony
Program of the 4th Asian Society of Veterinary Pathologists (ASVP)  
Conference and Annual Meeting of The Thai Association of  
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November 19-20, 2009 Bangkok, Thailand

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Canine Grade III Chondrosarcoma  

Lack of Adaptive Response of Gamma Radiation for Protection against Neutron-induced Teratogenesis  
H. Lee, J. Kim, M. Song, H. Seo, C. Moon, J. Kim, S. Jo, S. Kim  

Effect of HemoHIM on Ovarian Morphology and Expression of Nerve Growth Factor in Rats with  
Steroid-induced Polycystic Ovaries  
S. Kim, H. Lee, J. Kim, C. Moon, J. Kim, C. Bae, H. Park, U. Jung, S. Jo  

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H. M. Tun, M. Wongphatcharachai, T. Wisedchanwet, P. Kitikoon, A. Amonsin  

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Spermatotoxicity and Oxidative Stress of Epichlorohydrin in Sprague-Dawley Rats  

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T. Mamom

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K. Chatdarong, S. Sirivaidyapong, S. Srisuwatanasakul, P. Linharattanaruksa

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P. Tummaruk, K. Srisuwatanasagul

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Porcine Reproductive and Respiratory Syndrome Virus Antigen Detection in the Uterine Tissue of Gilts Correlated to the Antibody Titer
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S.K. Shin, B.M. Park, K.J. Na, B. Ahn

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Acute Dermal Toxicity Test of Colloidal Silver Nanoparticles
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Diagnostic Cytology in Veterinary Clinical Medicine
*H. Sakai*

Pathology of Hepadna Virus Infection in Humans and Animals
*K. Abe*

Suppression of Rabies Virus Propagation in Mice Brain by Intracerebral Immunization of Inactivated Virus
*Y. Sundun, S. Yano, K. Ochaa, T. Umemura*
Clinical Trials of Adipose Derived Stem Cells and Muscle Derived Stem Cells

Cytological Diagnosis of Neoplasia
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Introduction to the Principles and Practice of Veterinary Surgical Pathology
P.C. Stromberg

Mammalian Models for Studies of Transmission of Highly Pathogenic Avian Influenza A (H5N1) Viruses with Meat from Infected Poultry

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Adenoid Basal Cell Carcinoma in a Horse

Inflammatory Myofibroblastic Tumor in an Amazon Jaguar
Canine Grade III Chondrosarcoma

Y. H. Kim, B. I. Yoon, I. C. Park, H. H. Kwak, J. H. Han*

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Keywords: canine, chondrosarcoma, S-100

Introduction

Chondrosarcoma of the tumors originated in thoracic wall is the second most common primary bone tumor. However, when compared to osteosarcoma, chondrosarcoma is few and far between in the dogs. Chondrosarcoma can be divided into two sub-classes, skeletal and extraskeletal chondosarcoma. In cases of masses of the thoracic wall, the most common developing location is on the seventh rib and at the costal-chondral junction, but can also occur in the sternum. The most common clinical sign is a visible growing mass on the thoracic wall. This tumor frequently affects middle-aged to older, ranging from 5.9 to 8.7 years, and medium to large dogs, especially boxers, German shepherds, golden retrievers and various mixed breeds, being rare in small breeds. Golden retrievers were at a higher risk of developing chondrosarcoma than any other breeds. Chondrosarcoma is malignant tumor in which the neoplastic cells produce variable quantities of cartilaginous or fibrillar matrix. We report this case of chondrosarcoma metastasized to lymph node and lung in a dog.

Materials and Methods

A three-year-old, male, mixed dog was presented to the hospital at the school of veterinary because of a mass on the left axillary region. The plain thoracic radiograph revealed a huge well-defined mass of left thoracic cavity and lysis of second rib. The oppression of mass induced an abnormal position of left first, third, forth, and fifth rib. Necropsy findings revealed a firm, white and smooth circumscribed mass in the thoracic wall (approximately 13x15x17cm in diameter) and center of the mass was filled with gelatinous and necrotic tissues. Diffused small and white nodules were also found in lung. The representative tissues were collected in 10% phosphate buffered formalin and were processed for histopathological and immunohistochemical staining, for S-100 (rabbit polyclonal anti-S-100; DAKO Inc., Carpinteria, CA, USA; 1:500) using the avidin-botin complex method according to the manufacturer’s instructions.

Result and Discussion

This neoplasm revealed variably disorganized lobules of chondrocytes within a chondroid matrix. The chondrocytes of various sizes and shapes were irregularly dispersed within an abundant cartilage matrix. Binucleated cells sometimes were not only present, but mitotic figures were present in tumor tissue. Frequently, the neoplasm was subdivided by bundles of collagenous tissue septae. At the center of the lobules, the neoplastic cells were individualized or congregated within lacunae surrounded by amorphous basophilic chondroid matrix, whereas peripheral cells of the lobules were similar in mesenchymal precursors of cartilage without the lacunae. Some lacunae contained more than one cell. Histological feature of this case was in accordance with Grade III in that it showed marked pleomorphic nuclei, hypercellularity and spindle cell type in the peripheral zone as well as pulmonary metastasis. Cartilage matrix in the tumor stained deeply with alcin blue-PAS staining. Neoplastic cells were also evident in the parenchyma of the lung and cortex of mandibular and pulmonary lymph node with cartilage matrix formed. The liver underwent congestion and pigmentation about the central vein due to chronic passive congestion in the lung. An immunohistochemical analysis showed that S-100 do not only distributed consistently throughout both the cytoplasm and nucleus, with cytoplasm reacting more strongly, but neoplastic cells also demonstrated cytoplasmic immunoreactivity against vimentin in the thoracic wall, lymph nodes and lung. Based on the results, this case originating in rib was confirmed as canine chondrosarcoma which be metastasized to the lung and lymph nodes through lymph vessel. In the best knowledge, this is the first report of chondrosarcoma of grade III in dog in Korea.

Fig. 1 The VD radiograph (A). A huge mass of the left thoracic cavity. Tumor gross findings (B). Masses on both sides of thoracic wall.

Fig. 2 Neoplastic cells, have one to four nuclei, are in lacunae surrounded by cartilage matrix Primary tumor of rib. H&E (A). Matrix and nuclei of neoplasm were positive for Alcian blue-PAS staining (B) and for S-100 (C), respectively. Magnification: x 100.

References

Introduction

Although there are some reports on neutron teratology, there is little information on the adaptive response of gamma radiation for protection against neutron-induced teratogenesis. This study examined whether or not a low dose of gamma radiation can induce an adaptive response in mouse fetuses exposed to a subsequent dose of neutrons in vivo.

Materials and Methods

Pregnant ICR mice were exposed to a priming dose of 0.3 Gy (0.9 Gy/min) of gamma-rays on day 10.5 of gestation and challenged with 0.8 Gy (0.94 Gy/min) of neutrons 24 h later. The mice were sacrificed on day 18.5 of gestation. The fetuses were examined for mortality, growth retardation and other morphological abnormalities.

Table 1. Effects of Priming Dose at 0.3 Gy of Gamma-rays on Day 10.5 of Gestation Prior to a Challenging Dose at 0.8 Gy of Neutrons on Day 11.5 of Gestation on the Caesarean Section Findings in Mice

<table>
<thead>
<tr>
<th>Observations</th>
<th>Sham exposure</th>
<th>Gamma-rays (0.3 Gy)</th>
<th>Neutrons (0.8 Gy)</th>
<th>Gamma-rays (0.3 Gy) + neutrons (0.8 Gy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of mother</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>No. of implants</td>
<td>12.3±1.8</td>
<td>13.7±1.5</td>
<td>13.7±2.6</td>
<td>11.3±1.3</td>
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<tr>
<td>No. of embryonic death</td>
<td>0.50±0.55</td>
<td>0.17±0.41</td>
<td>0.16±0.40</td>
<td>0.33±0.50</td>
</tr>
<tr>
<td>No. of fetal death</td>
<td>0.33±0.52</td>
<td>0</td>
<td>0.83±1.17</td>
<td>1.17±0.98</td>
</tr>
<tr>
<td>No. of reorption</td>
<td>0</td>
<td>0.67±0.52</td>
<td>1.00±0.89</td>
<td>0.50±0.55</td>
</tr>
<tr>
<td>Prenatal mortality (%)</td>
<td>6.45±7.27</td>
<td>5.76±4.90</td>
<td>15.27±10.32</td>
<td>21.43±14.65</td>
</tr>
<tr>
<td>Live fetuses</td>
<td>11.50±1.52</td>
<td>12.82±0.98</td>
<td>11.67±1.75</td>
<td>9.33±4.13</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>1.59±0.09</td>
<td>1.51±0.08</td>
<td>0.85±0.08*</td>
<td>0.85±0.10*</td>
</tr>
<tr>
<td>Body length (cm)</td>
<td>3.45±0.63</td>
<td>3.48±0.42</td>
<td>2.53±0.12*</td>
<td>2.53±0.20**</td>
</tr>
<tr>
<td>Head length (cm)</td>
<td>1.15±0.05</td>
<td>1.14±0.04</td>
<td>0.78±0.03*</td>
<td>0.78±0.02**</td>
</tr>
<tr>
<td>Head width (cm)</td>
<td>0.84±0.02</td>
<td>0.80±0.03**</td>
<td>0.65±0.03*</td>
<td>0.65±0.05**</td>
</tr>
<tr>
<td>Tail length (cm)</td>
<td>1.25±0.03</td>
<td>1.10±0.05**</td>
<td>0.96±0.04*</td>
<td>0.84±0.16**</td>
</tr>
<tr>
<td>GRF No. (%)</td>
<td>5 (7.25)</td>
<td>(25.97)**</td>
<td>70 (100)**</td>
<td>56 (100)**</td>
</tr>
<tr>
<td>Fetuses with decreased head width (%)</td>
<td>2.90</td>
<td>25.97**</td>
<td>100**</td>
<td>100**</td>
</tr>
<tr>
<td>Fetuses with decreased head weight (%)</td>
<td>1.45</td>
<td>5.19**</td>
<td>100**</td>
<td>100**</td>
</tr>
</tbody>
</table>

The values are presented as means ± SD.

GRF: Growth retarded fetuses, calculated as the number of growth retarded fetuses/total number of live fetuses. Fetuses weighting less than two standard deviations of mean body weight of the control group were considered as growth retarded.

Table 2. Effects of Priming Dose at 0.3 Gy of Gamma-rays on Day 10.5 of Gestation Prior to a Challenging Dose at 0.8 Gy of Neutrons on Day 11.5 of Gestation on the Fetal Morphological Findings in Mice

<table>
<thead>
<tr>
<th>Observations</th>
<th>Sham exposure</th>
<th>Gamma-rays (0.3 Gy)</th>
<th>Neutrons (0.8 Gy)</th>
<th>Gamma-rays (0.3 Gy) + neutrons (0.8 Gy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>External malformation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fetuses examined</td>
<td>35</td>
<td>39</td>
<td>35</td>
<td>30</td>
</tr>
<tr>
<td>Fused thoracic centrum</td>
<td>0</td>
<td>0</td>
<td>2 (5.71)</td>
<td>0</td>
</tr>
<tr>
<td>Fused lumbar centrum</td>
<td>0</td>
<td>0</td>
<td>1 (2.86)</td>
<td>2 (7.69)</td>
</tr>
<tr>
<td>Absent caudal vertebra</td>
<td>0</td>
<td>0</td>
<td>2 (5.71)</td>
<td>2 (7.69)</td>
</tr>
<tr>
<td>Misshapen caudal centrum</td>
<td>0</td>
<td>0</td>
<td>2 (5.71)</td>
<td>0</td>
</tr>
<tr>
<td>Fused caudal centrum</td>
<td>0</td>
<td>0</td>
<td>13 (37.14)**</td>
<td>19 (73.08)**</td>
</tr>
<tr>
<td>Absent rib</td>
<td>0</td>
<td>0</td>
<td>1 (2.63)</td>
<td>3 (8.57)</td>
</tr>
<tr>
<td>Wavy rib</td>
<td>0</td>
<td>0</td>
<td>3 (8.57)</td>
<td>2 (7.69)</td>
</tr>
<tr>
<td>Short rib</td>
<td>0</td>
<td>0</td>
<td>1 (2.86)</td>
<td>0</td>
</tr>
<tr>
<td>Bent tibia</td>
<td>0</td>
<td>0</td>
<td>1 (2.86)</td>
<td>1 (3.84)</td>
</tr>
<tr>
<td>Bent tubia</td>
<td>0</td>
<td>0</td>
<td>1 (2.86)</td>
<td>0</td>
</tr>
<tr>
<td>Absent metatarsal</td>
<td>0</td>
<td>0</td>
<td>5 (14.29)</td>
<td>2 (7.69)</td>
</tr>
<tr>
<td>Absent metacarpal</td>
<td>0</td>
<td>0</td>
<td>4 (11.43)</td>
<td>3 (11.53)</td>
</tr>
<tr>
<td>Absent phalanx</td>
<td>0</td>
<td>0</td>
<td>17 (48.57)**</td>
<td>14 (53.85)**</td>
</tr>
<tr>
<td>Malformed offspring</td>
<td>0</td>
<td>2 (2.60)</td>
<td>47 (67.14)**</td>
<td>50 (89.29)**</td>
</tr>
</tbody>
</table>

Results and Discussion

The tail length in the 0.3 Gy of gamma-rays + 0.8 Gy of neutrons group was significantly shorter than in the 0.8 Gy of neutrons group. Although there was no significant difference compared with the 0.8 Gy of neutrons group, the number of live fetuses in the 0.3 Gy of gamma-rays + 0.8 Gy of neutrons group was lower. There was no evidence of primed exposure-related reductions in the malformed fetuses. Although there was no significant difference compared with the unprimed group, the number of malformed offspring in the primed group was higher. Furthermore, the incidence of kinked tail and adactyly was significantly higher in the primed mice than in the unprimed mice. Overall, this study shows that exposure to 0.3 Gy of gamma-rays failed to induce an adaptive response of fetogenesis to a neutron challenge dose.

Table 3. Effects of Priming Dose at 0.3 Gy of Gamma-rays on Day 10.5 of Gestation Prior to a Challenging Dose at 0.8 Gy of Neutrons on Day 11.5 of Gestation on the Fetal Morphological Findings in Mice

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<td>0</td>
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</tr>
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<td>0</td>
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</tr>
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<td>1 (2.86)</td>
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</tr>
<tr>
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<td>1 (3.84)</td>
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<tr>
<td>Bent tubia</td>
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<td>0</td>
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<td>0</td>
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<td>3 (11.53)</td>
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<tr>
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<td>0</td>
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</tr>
</tbody>
</table>

The values are presented as means ± SD.

GRF: Growth retarded fetuses, calculated as the number of growth retarded fetuses/total number of live fetuses. Fetuses weighting less than two standard deviations of mean body weight of the control group were considered as growth retarded.

A head width or length of less than two standard deviations of mean body weight of the control group were considered as growth retarded.

*Indicates a significant difference at p<0.05 compared with the sham exposure group.

**Indicates a significant difference at p<0.01 compared with the sham exposure group.

***Indicates a significant difference at p<0.001 compared with the sham exposure group.

**Indicates a significant difference at p<0.01 compared with the sham exposure group and neutrons (0.8 Gy) group.

Indicates a significant difference at p<0.05 compared with the sham exposure group.

Indicates a significant difference at p<0.01 compared with the sham exposure group and significant difference at p<0.05 compared with the neutrons (0.8 Gy) group.
Effect of HemoHIM on Ovarian Morphology and Expression of Nerve Growth Factor in Rats with Steroid-induced Polycystic Ovaries

S. Kim1*, H. Lee2, J. Kim1, C. Moon1, J. Kim1, C. Bae1, H. Park3, U. Jung3, S. Jo3

1College of Veterinary Medicine, Chonnam National University, Gwangju, South Korea 500-757 2Korea Institute of Radiological & Medical Science, Seoul, South Korea 139-706 3Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, Jeongeup, South Korea 580-185 4Corresponding author: shokim@chonnam.ac.kr

Key words: HemoHIM, nerve growth factor, polycystic ovary

Introduction
Estradiol valerate (EV)-induced polycystic ovaries (PCO) in rats cause the anovulation and cystic ovarian morphology. Presently, we investigated whether the treatment of HemoHIM influences the ovarian morphology and the expression of nerve growth factor (NGF) in an EV-induced PCO rat model.

Materials and Methods
PCO was induced by a single intramuscular injection of EV (4 mg, dissolved in sesame oil) in adult cycling rats. HemoHIM was either administered orally (100 mg/kg of bw/day) for 35 consecutive days or injected intraperitoneally (50 mg/kg of bw) every other day after EV injection.

Results and Discussion
Ovarian morphology was almost normalized and NGF was normalized in the PCO+HemoHIM group. HemoHIM lowered the high numbers of antral follicles and increased the number of corpus luteum in PCO ovaries. The results are consistent with a beneficial effect of HemoHIM in the prevention and treatment of PCO syndrome.

Table 1. Body and ovary weight measurements

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>Ovaries weight (mg) per g body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil control</td>
<td>244.5±15.5</td>
<td>0.43±0.08</td>
</tr>
<tr>
<td>PCO</td>
<td>226.3±19.9</td>
<td>0.27±0.03</td>
</tr>
<tr>
<td>PCO + HemoHIM1</td>
<td>232.2±13.4</td>
<td>0.32±0.04*</td>
</tr>
<tr>
<td>PCO + HemoHIM2</td>
<td>223.5±17.6</td>
<td>0.31±0.07</td>
</tr>
</tbody>
</table>

Table 2. Number of corpus luteum in the largest cross section of the ovary

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of corpus luteum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil control</td>
<td>11.2±3.2</td>
</tr>
<tr>
<td>PCO</td>
<td>2.4±1.3</td>
</tr>
<tr>
<td>PCO + HemoHIM1</td>
<td>5.0±2.2*</td>
</tr>
<tr>
<td>PCO + HemoHIM2</td>
<td>4.8±1.6**</td>
</tr>
</tbody>
</table>

Results are expressed as mean±S.D.
The Sprague-Dawley female rats (n=7) were injected with estradiol valerate (EV) and were autopsied 35 days later.

1HemoHIM (100 mg/kg of bw/day, p. o.) was given for 35 days after EV injection.
2HemoHIM (50 mg/kg of bw) was given i.p. once every other day for 35 days after EV injection.
*p<0.05 versus the PCO group.

Fig. 1 Ovarian morphology. (A) Oil control group. (B) Polycystic ovary (PCO) group. (C) PCO+HemoHIM 100 mg/kg (p.o.) group. (D) PCO+HemoHIM 50 mg/kg (i.p.) group. Hematoxylin and eosin stain, x 40. *depicts corpora lutea.

Fig. 2 Representative ovarian distribution of nerve growth factor by immunohistochemistry in the ovary tissue of an estradiol valerate-treated rat. Survey view (A) showing regressed corpus lutea, atretic follicles, and healthy growing follicles. Detailed view (B) of a healthy growing follicle (framed right area in A). Detailed view (C) of a regressed corpus luteum (framed middle area in A). Detailed view (D) of an atretic follicle (framed left area in A).

Fig. 3 Nerve growth factor (NGF) protein levels in the ovaries by western blot analysis. Ovary tissue from polycystic ovary (PCO) rats contained significantly higher levels of ovarian NGF protein than oil control ovary tissue. The level of NGF protein in ovary samples from PCO + HemoHIM rats was significantly lower than in samples from PCO rats. The relative optical densities (OD) of NGF expressions were determined by densitometry and normalized to the β-actin signals from three different samples. Values for oil controls were arbitrarily defined as 1. Values are given as mean ± S.E. *p<0.05, **p<0.01 versus the PCO group.
Differential CARM1 Expression in Prostate and Colorectal Cancers

Y.R. Kim1, B.K. Lee2, R.-Y. Park1, N.T.X. Nguyen1, D.D. Kwon3,4, C. Jung1,4*

1Departments of Anatomy, 2Emergency Medicine, 3Urology, 4Research Institute of Medical Sciences, Chonnam National University Medical School, Gwangju, Korea  *Corresponding author: chjung@chonnam.ac.kr

Keywords: CARM1, colorectal cancer, prostate cancer

Introduction

Coactivator-associated arginine methyltransferase 1 (CARM1) functions as a transcriptional coactivator mainly studied in its association with nuclear hormone receptors. CARM1 showed significant role in androgen-stimulated androgen receptor (AR)-mediated transactivation. Recently, overexpression of CARM1 was shown to be involved in the development of prostate cancer (PCa), including androgen-independent PCa.

Materials and Methods

An attempt to profile an expression pattern of CARM1 in human cancers, tissue microarray was utilized to immunolocalize CARM1. To confirm the expression of CARM1, surgical specimen with full clinical data were further utilized, including colorectal cancers and androgen-refractory prostate cancers. To validate CARM1’s action mechanism in non-hormone mediated signaling pathway, reporter transcription assay was employed in various cancer cells.

Results and Discussion

Tissue microarray showed that CARM1 was particularly overexpressed in colorectal cancers while CARM1 expression was not prevalent in other tumors, including prostate and breast cancers (Table 1). Further studies using surgical specimen demonstrated that CARM1 was highly overexpressed in 75% of colorectal cancers (38 out of 54) (Fig. 1A) but not in androgen-independent PCa compared to androgen-responsive tumors (Fig. 1B). In addition, CARM1’s coactivating function in AR-mediated transactivation was very minimal in androgen-independent cells. Further assays using PSA promoter showed minor coactivating function in both androgen-dependent and androgen-independent cells implying that there may be other factors that CARM1 affects (Fig. 2A-B). Correspondingly, CARM1 showed either suppressive or promoting roles in p53 and NF-kappaB target gene transcription, respectively (Fig. 2C-D). This study suggests that, in addition to its role in hormone receptors, CARM1 may function as transcriptional modulator for other growth regulators through non-AR mediated transactivation in androgen independent PCa and colorectal cancers. However, CARM1’s action of mechanism needs to be further clarified.

Table 1. Score of CARM1 expression in human tumors

<table>
<thead>
<tr>
<th>Tumor types</th>
<th>Score of CARM1 expression*</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>D=1**</th>
<th>2=3**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain tumor</td>
<td></td>
<td>22</td>
<td>10</td>
<td>0</td>
<td>32</td>
<td></td>
<td>5 (8 %)</td>
</tr>
<tr>
<td>Melanoma</td>
<td></td>
<td>25</td>
<td>5</td>
<td>2</td>
<td>30</td>
<td></td>
<td>5 (14 %)</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td></td>
<td>22</td>
<td>7</td>
<td>5</td>
<td>29</td>
<td></td>
<td>6 (17 %)</td>
</tr>
<tr>
<td>Lymphoma</td>
<td></td>
<td>50</td>
<td>8</td>
<td>0</td>
<td>67</td>
<td></td>
<td>9 (12 %)</td>
</tr>
<tr>
<td>Lung cancer</td>
<td></td>
<td>61</td>
<td>25</td>
<td>0</td>
<td>26</td>
<td></td>
<td>10 (19 %)</td>
</tr>
<tr>
<td>Breast cancer</td>
<td></td>
<td>31</td>
<td>22</td>
<td>13</td>
<td>5</td>
<td></td>
<td>20 (27 %)</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td></td>
<td>16</td>
<td>17</td>
<td>59</td>
<td>31</td>
<td></td>
<td>70 (68 %)</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td></td>
<td>85</td>
<td>17</td>
<td>5</td>
<td>2</td>
<td>102</td>
<td>7 (6 %)</td>
</tr>
</tbody>
</table>

* CARM1 expression was scored as follows: 0, no; 1, low; 2, moderate; 3, high. **Scores were categorized by D as normal expression and 2 as overexpression

Fig. 1 Immuno-localized CARM1 was scored and demonstrated as either bar graph (A) or scattered plot (B). A. colorectal cancers, B. prostate cancers; *denoted p<0.05; AD, androgen dependent; AI, androgen-independent.

Fig. 2 CARM1 regulates p53- and NF-kappaB mediated response in addition to androgen-stimulated PSA response. Reporter transcription assay was performed in LNCaP cells with cotransfection of CARM1 and indicated luciferase DNA. Bar is shown as mean ± SD. P61-luc uses whole PSA promoter.
One Step Reverse Transcription Loop-mediated Isothermal Amplification for Influenza A Rapid Detection

H. M. Tun¹, M. Wongphatcharachai¹, T. Wisedchanwet¹, P. Kitikoon², A. Amonsin*¹

¹Department of Veterinary Public Health, ²Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand 10330 *Corresponding author: heinmin@gmail.com

Keywords: influenza A, loop-mediated isothermal amplification, reverse transcription

Introduction

Influenza viruses are segmented negative-sense single strand RNA viruses that belong to the family Orthomyxoviridae. The influenza viruses can be divided into types A, B or C and type A influenza virus can further divided into different subtypes according to its surface glycoproteins, i.e. hemagglutination (HA) and neuraminidase (NA). Among these 3 types, Influenza A viruses infect humans, pigs, horses, seals and whales as well as a variety of domestic and wild birds (5). It is generally accepted that in the human influenza pandemics and numerous outbreaks in domestic and wild animals, interspecies transmission of Influenza A viruses have played a crucial role (2). Three times during the last century, influenza A viruses caused major pandemics, i.e. “Spanish flu” 1918, “Asian flu” 1957 and “Hong Kong flu” 1968, with a high mortality rate all over the world (6). And also recently the new strain of Influenza A virus caused the pandemic flu so called “Pandemic H1N1” 2009 (7). Therefore, a lot of surveillance networks had been established and were still establishing for the next influenza pandemic. For the influenza A, the viral isolation technique (VIT) is still as a gold standard diagnostic tool. But this method can be performed only by specialized laboratories, and 3 to 10 days is required for the availability of the results. Some rapid detection and easy to perform antigenic detection tests are helpful such as immunofluorescence (IFA) assays (4) and reverse transcription-PCR (8). However IFA assays have limited sensitivity compared to VIT and also need of sophisticated equipments for the application of PCR techniques. A choice of diagnostic technology can now be based on a combination of factors that includes fitness-for-purpose, technical ease, speed, sensitivity, specificity and cost (1). The loop-mediated isothermal amplification (LAMP) assay is sensitive and easy to perform when compared with reverse transcriptase-PCR. In addition, the assay is not required any expensive special equipment, such as thermal cycler. Therefore, the LAMP method would be suitable for the mobile surveillance and for unequipped laboratories in developing countries (3). In this study, we developed one step RT-LAMP technique for rapid detection of influenza A viruses.

Materials and Methods

Designing the RT-LAMP primers: The LAMP primers were designed using Primer Explorer V3 software based on a conserved region of M gene identified by sequence alignment (Fig. 1).

RNA standard for detection limit (sensitivity) and specificity test: Influenza A virus (A/chicken/Thailand/CU-K2/2004 (H5N1)) was used for sensitivity test as reference virus. Total RNA was extracted from 140 μl of allantoic fluid using QIAamp Viral RNA Mini Kit (Qiagen GmbH, Hilden) according to the manufacturer’s specifications. The concentration of the RNA was measured by NanoDrop1000. The RNAs were then serially diluted 10-fold. The specificity of RT-LAMP was tested against other RNA viruses caused animal diseases which are porcine reproductive and respiratory syndrome virus (PRRSV), classical swine fever virus (CSFV), Infectious bronchitis virus (IBV) and Newcastle disease virus (NDV).

RT-LAMP detection: The LAMP reaction was carried out in a volume of 25 μl containing 1x ThermoPol buffer (NEB, USA), 0.4mM dNTPs, 8mM of MgSO₄, 0.2 μM each of F3 and B3, 1.6 μM of FIP and BIP, 0.04 M betaine (Sigma), 5U cloned AMV reverse transcriptase (Invitrogen, USA), 8U Bst polymerase (NEB, USA) and 2 μl of RNA. To optimize the LAMP assay condition, an evaluation was
taken on the effects of reaction time (30-70 minutes) and reaction temperature (60-65°C) and then for 10 minutes at 80°C to terminate the reaction. All amplification steps were taken by using water bath.

Results and Discussion

Optimal condition for RT-LAMP assay: The effects of temperature (60, 63, 65°C) and time (30, 40, 50, 60, 70 minutes) were tested. The optimal condition for RT-LAMP assay was determined to 63°C for 1 hour then followed by 80°C for 10 minutes (Fig. 2).

Detection limit of RT-LAMP assay: The detection limit of RT-LAMP on M genes was 1.7 picograms of RNA which is equivalent to Ct 36 in real-time RT-PCR assay and also 10 times more sensitive when compared with conventional one step RT-PCR (17 pg).

Specificity of RT-LAMP assay: The specificity of RT-LAMP was tested against other veterinary RNA viruses and the result shown that high specificity of LAMP primers to influenza A virus.

In the present study, we developed a rapid and high sensitive influenza A detection system without the need of highly expensive equipment. This RT-LAMP assay was highly specific to influenza A and 10 times more sensitive than ordinary one step RT-PCR assay. In the future, we expected to apply this developed RT-LAMP assay to test the clinical samples and apply in the field together with easy RNA preparation where minimal laboratory facilities were provided.

Acknowledgements

We would like to thank the staffs from Veterinary Diagnostic Laboratory, Faculty of Veterinary Science, Chulalongkorn University for their assistant.

Reference

Lectin Histochemistry Assay in Colon Tissues for Characterization of Rodents

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*Corresponding author: ranria77@yahoo.com

Keywords: colon, glycoconjugate, histochemistry, lectins, rodents,

Introduction

Lectins are ubiquitous proteins of non-immune origin which are present in animals and humans. Lectins bind specifically monosaccharides or oligosaccharide structures (1). The digestive tract of animals including rodents has taxonomic and ecologic importance due to its ability in adaptation for different environments. Whereas colon absorbs water and carbohydrates; it has a vital role ecologically and physiologically and enjoys different histological structures in different rodents with various diets (2). Nowadays, Wheat Germ Agglutinin (WGA), Peanut Agglutinin (PNA) and Concanavalin (Cona) are being used widely for studying sugar compounds on the surface of cells (3). Few studies have investigated glycoconjugates in the gastroenteric mucosa by lectin histochemical methods. The aim of this current study is to investigate the characteristic distribution of certain glycoconjugates that exist in the colon of various species of rodents by means of lectin histochemistry for phylogenic characterization.

Materials and Methods

Ten different species of rodents that belong to different families were selected. The involved rodents were belonging to many families including the family of Muridae; consisting of Gerbilinae subfamily (Jerbillus nanus, Meriones Persicus, Meriones lybicu) Microtinae (Microtus transcaspicus, Ellobius fasscocapillus, Microtus sp) Dipodidae (Alactage elater, Jucullus blanfordi) and scuridae (Funambulus penantii, spermophillus folvus). The digestive tract of all involved rodents was removed properly and weight was measured and then the proximal colon was cut into 2-3 cm segments, fixed by Bouin solution and 4 μm thick paraffin-embedded sections were made and sections were stored at 4°C for later use. Lectin histochemistry assay was conducted.

Lectin histochemistry assay: Lectin binding was performed as previously reported by Ferri et al (4). Briefly, two horse-raddish peroxidase (HRP)-conjugated lectins were used (Sigma, USA), PNA and WGA. 200 μg of lectins were diluted in 800 μl of 0.1M Phosphate-Buffered Saline (PBS). Two sections of colon were chosen and then hydrated and rinsed for 10 min in 0.1M PBS (pH 7.4). Rehydrated sections were exposed to 3% H2O2 for 10 min and then incubated for 30 min at 20°C with peroxidase-labeled lectin in 0.1M PBS. The activity of the HRP was then visualized histochemically by adding 2-3 drops of 0.005% 3-3-diaminobenzidine (DAB) (Sigma, USA) All sections were counterstained with 1% solution of Alcian Blue at pH 2.5 for 5 min. The intensity of the labeling was classified as shown in (Table 1).

Table 1 The scoring system used for assessing the staining intensity of lectin binding assay (5)

<table>
<thead>
<tr>
<th>Description</th>
<th>Grades</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative reaction</td>
<td>-</td>
</tr>
<tr>
<td>Weak</td>
<td>+</td>
</tr>
<tr>
<td>Moderate</td>
<td>++</td>
</tr>
<tr>
<td>Severe</td>
<td>+++</td>
</tr>
<tr>
<td>Very severe</td>
<td>++++</td>
</tr>
</tbody>
</table>

Results and Discussion

Microscopic observations indicated that there were remarkable distinctions in the carbohydrates and glycoproteins content of the different layers of colon among different species of rodents. Both PNA and WGA lectin histochemistry showed high and significant discriminatory power among the studied species of...
Fig. 1 (Upper left) transverse sections of proximal colon in Funumbulus pennantii (F.p) in reaction with PNA lectin at magnification 100X. Gc: Goblet cells showed no lectin reaction. (Upper right) transverse sections of proximal colon in Meriones lybicus (M.l) in reaction with WGA lectin at magnification 100X. Gc: Goblet cells, severe reaction was observed. (Lower left) transverse section of proximal colon in Ellobius fascocapillus incubated with WGA at magnification 100X. S: Serosa layer with very severe reaction, Ol: Outer longitudinal muscle layer with severe reaction, Ic: Inner circular muscle layer with weak reaction, Sb: submucosal layer with moderate reaction, Gc: Goblet cells with no reaction, A: Absorptive cells with moderate reaction. (Lower right) transverse sections of proximal colon in Alactage elater (A.e) with PNA lectin at magnification 100X. Sb: submucosal layer, moderate reaction. S: Serosa layer, Ol: Outer longitudinal muscle layer, and Ic: Inner circular muscle layer, Gc: Goblet cells all showed no reaction with lectin.

rodents especially lectin staining in mucosal and absorptive cells (p<0.05) (Fig. 1). Goblet and epithelial cells showed much lower affinity to lectins. No reaction of PNA was observed in longitudinal muscle layer, circular muscle layer, goblet cells in channel and in surface of most studied rodents. Weak to moderate reaction of PNA was observed in other layers of colon. Severe reaction was observed in serosa layer of all studied rodents except dipodidae family Alactage elater and Jaculus blanfordi. Weak to strong reaction of WGA was observed in different layers of colon of rodents except in goblet cells in channel, basement and surface epithelium compartments of most rodent species. It was concluded that the use of lectin histochemistry is a valid method for the phylogenetic characterization of rodents, may be other animals, depending on colon tissues. Moreover, colon tissues proved to be highly distinct and variable among rodent species. Therefore, this assay can also be used in determining the diet nature, geographical variation, diseases affection on different species of animals.

References
Pathological Investigation of Reproductive Organs of Culled Boars in Thailand

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Keywords: atrophy, boar, degeneration, fibrosis, testis

Introduction

Infertility of boars is an important factor for culling and leads to an economic loss. Pathological changes of reproductive organs of boars result in poor semen quality and infertility. Various lesions of infertile/subfertile boars have been documented including testicular degeneration, hypoplasia, segmental aplasia of Wolffian ducts\textsuperscript{1} and inflammation. In Thailand, the data concerning pathology of culled boars are inadequate; therefore, this slaughterhouse-based study was conducted. The objective of this study was to determine the prevalence of pathological changes of testes of culled boars.

Materials and Methods

The genital organs of boars (n = 100) from slaughter houses were pathologically examined. For histopathology, 3 sections of each testis were fixed in 10\% formalin and the adjacent sections were put in Bouin’s solution.

Results and Discussion

The culling causes included infertility (n = 30), old age (n = 9), leg problems (n = 13), scrotal enlargement (13\%, n = 13), scrotal enlargement with leg problems (n = 2), other problems (n = 8), and the remaining had no information. The pathological findings were summarized in Table 1.

In the present study, testicular fibrosis was the most frequent lesion (n = 85), mainly occurred in mild and moderate degree. This lesion was usually accompanied with degeneration, but sometimes it could occur without degeneration. Testicular fibrosis could be the consequence of myoid cells alteration into myofibroblast\textsuperscript{2}. The cause of the lesion is unknown but it might be associated with viral infection in cattle\textsuperscript{3}.

The second most frequent lesion found in the testes was various degrees of seminiferous tubule degeneration (n = 73). The mechanism of degeneration is related to apoptosis of germ cells, which might be the results of toxic substance or viral infection(1,4). Interestingly, small-sized testes so called testicular atrophy were often observed (n = 20) (Fig 1). The lesion was sometimes associated with various degrees of non-suppurative interstitial orchitis,

Table 1. Lesions of reproductive organs of culled boars

<table>
<thead>
<tr>
<th>Lesions</th>
<th>Numbers / Severity</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testicular fibrosis</td>
<td>36 28 21</td>
<td>85</td>
</tr>
<tr>
<td>Testicular degeneration</td>
<td>25 29 19</td>
<td>73</td>
</tr>
<tr>
<td>Testicular atrophy</td>
<td>5 3 12</td>
<td>20</td>
</tr>
<tr>
<td>Suppurative orchitis</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Non-suppurative interstitial orchitis</td>
<td>24 12 3</td>
<td>39</td>
</tr>
</tbody>
</table>

+: mild, ++: moderate, +++: severe

Fig. 1 Severe testicular atrophy of right testis (A), Bar = 1 cm. Histology of Fig. 1 A showing atrophic seminiferous tubules (S), with interstitial orchitis. (B), Bar = 25 \( \mu \)m

fibrosis and degeneration. However, the etiology of the lesion is still unclear.

In conclusion, the results suggested that testicular fibrosis and degeneration were the two most frequent lesions found in the reproductive organs of culled boars in Thailand. Though, the causes of these lesions could not be definitely determined, but clinical examinations and pathological findings could indicate the causes of infertility.

Acknowledgements

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References

Cyclophosphamide Induces Deficit for Hippocampus-dependent Learning and Memory

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Keywords: cognitive impairment, cyclophosphamide, hippocampus, neurogenesis

Introduction

Cyclophosphamide (CYP), a cytotoxic alkylating agent, is commonly used as an antineoplastic agent for the treatment of various cancers, as well as an immunosuppressive agent for organ transplantation, systemic lupus erythematous and other benign diseases.

Cognitive impairment occurs in a subset of cancer survivors and is generally subtle. Most evidence suggests an association with chemotherapy although other factors associated with the diagnosis and treatment of cancer may contribute.

In this study, we examined the behavioral change of adult mice after CYP injection by hippocampus-dependent learning paradigms. In addition, the effect of cancer chemotherapy on hippocampal neurogenesis was investigated by examining the changes in the number of DCX (immature cell marker)- and Ki-67 (proliferating cell marker)-positive cells in the dentate gyrus (DG) of hippocampus of adult mice after administration of CYP.

Materials and Methods

The behavioral dysfunction in the mice after receiving an intraperitoneal injection of CYP (40 mg/kg) was measured by open field analysis (n = 8 mice/group), passive avoidance (n = 9 mice/group), and object recognition memory test (n = 9 mice/group) at 12 h and 10 days after injection, respectively.

To elucidate the effects of CYP on neurogenesis in the adult mouse hippocampus after receiving an intraperitoneal injection of CYP (40 mg/kg), the mice were sacrificed and the hippocampi were then dissected from each group at 12, 24 h, 3 and 10 days (n = 6 mice/group) after CYP treatment.

Results and Discussion

CYP-treated mice showed normal locomotor activity. Compared to vehicle-treated controls, mice were trained 12 h after CYP treatment induced a deficit in memory of passive avoidance task and object recognition task tested 24 h after training, but mice 10 days after CYP treatment did not show any memory defect.

The Ki-67- and DCX-positive cells in the DG were decreased significantly by 6-24 h after the CYP injection (40 mg/kg), and then recovered to normal levels similar to the non-treatment controls.

In conclusion, we suggest that a cancer chemotherapeutic agent, cyclophosphamide, is sufficiently detrimental to interrupt the functioning of the hippocampus including learning and memory, possibly through the inhibition of neurogenesis.

Acknowledgment

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Spermatotoxicity and Oxidative Stress of Epichlorohydrin
in Sprague-Dawley Rats


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Keywords: epichlorohydrin, oxidative stress, rats, reproductive dysfunction, sperm

Introduction
Epichlorohydrin (ECH) is one of the industrial chemicals used in the manufacture of glycerol, epoxy resins, and other products. Due to its increased production and widespread use, the human exposure to ECH has steadily increased, which may result in severe health impacts.

Previous studies demonstrated that ECH is an anti-fertility agent that acts both as an epididyimal toxicant and an agent capable of directly affecting sperm motility. Although it is suggested that the anti-fertility effects of ECH maybe resulted from reduced sperm motility and sperm metabolism, the mechanism underlying the ECH-induced sterility has not been fully elucidated.

In this study, we examined the spermatotoxicity and epididyimal oxidative damage of ECH after repeated oral administration in male rats to better understand a possible mechanism for the spermatotoxicity of ECH.

Material and Methods
Male rats aged 6 weeks were administered ECH daily by gavage at 0, 3.3, 10, and 30 mg/kg/day for 10 weeks and sacrificed 24 h after the last administration of ECH. Spermatotoxicity was assessed by measurement of reproductive organ weights, testicular spermatid count, epididymal sperm count, motility and morphology, and histopathology. Oxidative stress was assessed by the measurement of malondialdehyde (MDA), reduced glutathione (GSH), catalase, superoxide dismutase (SOD), and glutathione-S-transferase (GST) in epididymis.

Results and Discussion
General findings: The high dose group showed treatment-related clinical signs including nasal discharge (n=4), soft feces (n=1), depression (n=2), and piloerection (n=3). The number of animals with clinical signs (n=7) was significantly increased when compared with the control group. There were no statistically significant differences in the body weight and food consumption between the groups.

Autopsy findings: Cystic pustule of the epididymidis was observed in 5 cases of the high dose group. However, the incidence of gross pathology finding observed in the group was not significantly different compared with the controls. No significant difference between the groups was seen for any reproductive organ weight measured.

Sperm findings: Testicular spermatid count, epididymal sperm count, and sperm motility in the high dose group were significantly decreased in a dose-dependent manner compared with those of the control group. On the contrary, sperm morphological abnormalities including folded tail, short tail and no tail in the high dose group was significantly increased in comparison to the control group.

Histopathologic findings: There was a significantly higher incidence of histopathological findings in the 30 mg/kg group than the control values. Spermatic granuloma (n=6), cell debris in the ducts (n=12), epithelial cell desquamation (n=6), epithelial cell vacuolization (n=9), and oligospermia (n=3) were observed in epididymis of the 30 mg/kg group. Cell debris in the ducts (n=3) and epithelial cell vacuolization (n=6) were also found in the 10 mg/kg group.

Oxidative stress: The concentration of MDA in the 10 and 30 mg/kg groups was significantly increased in a dose-dependent manner when compared with the control group. On the contrary, the concentration of GSH was significantly decreased in all of treatment groups when compared with the control group (Fig. 3). The activities of catalase, GST, and SOD in the 10 and 30 mg/kg groups were also significantly lower than controls.

The increased incidence of cystic pustule was considered to be treatment-related effect, since this finding is uncommon in normal control rats and is consistent with the significantly increased incidence of histopathological alterations. Histopathologic changes observed in the present study included spermatic granuloma, cell debris in the ducts, desquamation of the epithelial cells, vacuolization of the epithelial cells, and oligospermia in the epididymis.

The significant decrease of testicular spermatid count, epididymal sperm count, and sperm motility and the significant increase of sperm abnormalities observed in the high dose group were also considered to be related to the ECH administration because these changes were remarkable and showed a clear-cut dose-response relationship.

The animals treated with ECH showed decreased activities of antioxidant enzymes catalase, GST, and SOD and GSH concentration, while increased concentration of MDA in the epididymis in a dose-related manner. Increased lipid peroxidation and reduced levels of antioxidants of epididymis in rats treated with ECH may indicate an increased ROS generation and could be closely linked to its effect on the epididymal and sperm indices.

It can be concluded that administration of ECH to male rats at ≥10 mg/kg/day elicits spermatotoxicity and oxidative damage in the epididymis and that the adverse effects of ECH on spermatotoxicity may be at least partially due to the induction of oxidative stress in the epididymis.

Acknowledgment
This work was supported by the Regional Research Centers Program (Bio-housing Research Institute), granted by the Korean Ministry of Education, Science and Technology.
Postmortem Radiographic Diagnosis of Pneumothorax in Dogs

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Keywords: dog, pneumothorax, postmortem, radiography

Introduction
Commonly used technique of incision into the thoracic cavity during necropsy is a major factor in the reduced ability to clearly diagnose pneumothorax at postmortem in dogs (3). Two-D X-radiography has been shown to be of high sensitivity and specificity in its clinical diagnosis, but have not been employed for such at necropsy (2). Advances have been made in forensic radiology in human autopsy leaving a wide gap to fill in the veterinary practice (1, 4). This study assesses the ability of diagnosing pneumothorax at postmortem examination using relatively simple and inexpensive X-radiography.

Materials and Methods
Five euthanized laterally recumbent canine carcasses were injected with 300-700 ml of air using 18G 20 ml syringe (Terumo®, Philippines) through the 7th and 8th intercostals space. Lateral (right and left) and ventrodorsal radiographic views of the carcasses were taken at 0, 60 and 120 min. Radiographs were read and interpreted blindly by UPM VTH radiologists to generate a table for sensitivity and specificity. Severity of lesion was classified as severe, moderate or mild if it spans over 2/3rd, 2/3rd or 1/3rd of the rib length, respectively.

Results and Discussion
A 100% sensitivity and specificity was recorded in the ability of radiography to diagnose pneumothorax at postmortem with (table 1) or without anamnesis (table 2). Anamnestic back up for the radiographs did not appear to have any influence on the interpretation of the radiographs for pneumothorax (Fig 1, 2). This may be due to the obvious gas opacity observable in the thoracic cavity as well as the leafing of the lungs signifying compression and collapse (Fig 3). Severity of the lesion was diagnosed based on 100% sensitivity with 63% of the radiographs being interpreted as severe, 27% moderate and 10% as mild (Table 3). These values corresponded with 300 to 400 ml of air for mild, 400 to 500 ml for moderate and 500 to 700 ml for severe. The crossover of values resulted from the method of measuring and interpreting the severity of the lesions. The mean radiolucent span of the rib length was

| Table 1 Diagnosis of postmortem pneumothorax with/ without anamnesis |
|---------------------------------|-----------------|-----------------|---------------|
|                                | Positive | Negative | Total |
| Pneumothorax +                 | 45       | 0        | 45    |
| Pneumothorax -                 | 0        | 45       | 45    |

| Table 2 Diagnosis of postmortem pneumothorax with/ without anamnesis |
|---------------------------------|-----------------|-----------------|---------------|
|                                | Positive | Negative | Total |
| Pneumothorax +                 | 45       | 0        | 45    |
| Pneumothorax -                 | 0        | 45       | 45    |

| Table 3 Measurement of severity of pneumothorax from 45 radiographs |
|---------------------------------------------------------------|-------|
| Pneumothorax (%)                                             |
| Severe                                                       | 63    |
| Moderate                                                     | 27    |
| Mild                                                         | 10    |
Fig. 1 Showing gas opacity on left lateral view.

Fig. 2 Showing gas opacity on ventrodorsal view

Fig. 3 showing collapsed lung at postmortem examination

taken for right and left lateral and ventrodorsal views, subjecting the values to variations effected by radiographic positioning causing fluctuations in the location of air in the thoracic cavity. Gross necropsy did not reveal a positive diagnosis of pneumothorax except for decreased negative pressure on puncture of the diaphragm.

Postmortem radiographic diagnosis of pneumothorax is 100% specific and sensitive whereas positioning for such may affect the interpretation of severity of lesion, however the positioning technique does not require a specialist. Routine survey postmortem radiography of the thorax is valuable in the accurate diagnosis of pneumothorax in dogs.

References
The Effects of Gemifloxacin on Achilles Tendon in Immature Rats

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Introduction

Gemifloxacin is one of the recently developed fluoroquinolones. It is a potent, novel quinolone with broad-spectrum antibacterial activity against both Gram-negative and Gram-positive pathogens. The side effect profiles such as diarrhea, rash, nausea, and headache are similar to those of the older members of this class.

Tendopathy is one of the major adverse effects caused by quinolone antibacterial agents, but the incidence of tendopathy is generally low at the clinical doses of the quinolones used. This study examined the effects of gemifloxacin on the Achilles tendon in immature rats with ofloxacin and ciprofloxacin used as a comparison. A relatively high dose of quinolones, which can induce tendopathy and/or arthropathy in juvenile rats, was used in this study.

Materials and Methods

The test chemical, gemifloxacin (Factive, LG Life Sciences Ltd., Daejon, Korea) was dissolved in a saline solution and ofloxacin and ciprofloxacin were suspended in a saline solution and administered by gavage. The individual dose volume (10 ml/kg bw) was determined according to the body weight immediately before administration.

The rats were treated once daily for 5 days by oral intubation with gemifloxacin at 0 (vehicle), and 600 mg/kg body weight. The animals were treated from postnatal day 30 to day 34 before being sacrificed, and samples were collected within 24 h after the final dose. Other rats of the same age were treated with either ofloxacin or ciprofloxacin at 600 mg/kg (once daily for 5 days).

The Achilles tendon samples were prepared from the right foot of five rats from each dosage group. Tangential sections were made from the distal part of the tendon. The specimens were examined by transmission electron microscopy (TEM, Hitachi H-7600, Japan).

Results and Discussion

In comparison with the vehicle-treated controls, there were ultrastructural changes in all samples from the gemifloxacin, ofloxacin-, and ciprofloxacin-treated rats. Degenerative changes were observed in the tenocytes, and the cells that detached from the extracellular matrix were recognizable.

The degree of degenerative changes and the number of degenerated cells in the Achilles tendon were significantly higher in the treated group than in the control group. Moreover, among the quinolone-treated groups, these findings were most significant in the ofloxacin-treated group, and least significant in the gemifloxacin-treated group.

Quinolones are antibacterial agents that have the potential to cause Achilles tendon disorders such as tendinitis or even ruptures. Quinolone treatments are contraindicated in juveniles and are only used in pediatrics in rare cases. A number of toxicological studies have confirmed that quinolone-induced tendopathy is a drug-induced, dose-dependent toxic effect of these agents. Kato et al. described quinolone-induced tendopathy after the single oral administration of pefloxacin or ofloxacin. Tendon lesions were induced in immature rats (4 wks of age) but not in 12-week-old rats. Tendon lesions were inhibited by the co-administration with dexamethasone and N-nitro-L-arginine methyl ester. In contrast, catalase, indomethacin, pyrilamine, and cinmetidine did not modify these tendon lesions. This suggests that nitric oxide and 5-lipoxygenase products partly mediate fluoroquinolone-induced tendon lesions.

Recent experiments have shown that ultrastructural alterations in tenocytes can be observed in immature and adult rats after being treated with quinolones. These effects were more pronounced when the animals were simultaneously given a magnesium-deficient diet, suggesting that the pathophysiology of tendopathy resembles that of arthropathy. When Shakibaei & Stahlmann examined the Achilles tendons from the quinolone-treated adult rats by electron microscopy 4-12 wks after treatment with single oral doses of ofloxacin, levofloxacin or fleroxacin, they could detect specific, pathological alterations already at the lowest dose (30 mg/kg), which increased in severity with increasing dose. The tenocytes detached from the extracellular matrix and showed degenerative changes such as multiple vacuoles and large vesicles in the cytoplasm, which resulted from swelling and dilatation of the cell organelles. Other findings were a general decrease in the fibril diameter and an increase in the distance between the collagenous fibrils.

The effects of gemifloxacin are of special interest because of the low chondrotoxic potential. It is unclear how ultrastructural changes in the Achilles tendons from immature rats relate to the potential risk in juvenile patients treated with gemifloxacin. However, these results underline the fact that, in principle, this new fluoroquinolone with a pyrrolidine derivative at the C-7 position has less potential to cause changes in the connective tissue structures. Further toxicological and clinical studies will be needed to characterize the conditions under which quinolone-induced tendon lesions develop.
Comparative Histological and Histochemical Inter-species Investigation of Mammalian Submandibular Salivary Glands

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Keywords: histochemistry, histology, mucin, rodents, salivary, submandibular

Introduction

Salivary glands have an important role in terrestrial animals, provide lubrication for eating and vocalization, aide digestion and supply saliva for pH buffering (1). Salivary glands of rodents are important elements regarding their adaptations to different diets, environments, and taxonomic studies (2). To reach a delicate analysis between biology and ecology of different animals, such as rodents, there is a need to study the salivary glands histology and histochemistry. Mucosal units react strongly with staining techniques, Alcian blue (AB) and PAS. Hence, this was exploited to conduct a histochemical interspecies analysis of submandibular salivary glands in rodents as a representative for mammalian animals.

Materials and Methods

The specimens of Funambulus pennati from Scuridae, Cricetulus migratorius, Meriones libycus, Mus musculus, Nesokia indica and Apodemus sp. from Muridae and two specimens from Dipodidae family Allactaga elater and Jaculus blanfordi were collected from different regions of Khorasan (Tandoureh Park, Moghan, Gonabad, Birjand and Kashmar). The whole skull was selected because it is hard to locate definitely the submandibular glands. Moreover, histological autopsy may harm the gland tissue. After washing blood off the heads with normal saline, they were placed in separate labeled buckles containing bouin’s fixative. Tissue preparation histology process, microtomy and staining were done. Staining was done with hematoxylin-eosin, tetrachrome, PAS alcian blue (pH=1) and PAS alcian blue (pH=2.5). The slides of all species were studied and compared with each other.

Results and Discussion

Microscopic histological features, including existence of mucus and serous acini, presence of different kinds of tubules, and different types of ducts were shown of valuable discriminatory value. In detail, distinctive and characteristic features of the histological investigations were found regarding mucus glands, their ducts, serous gland ducts, the presence or absence of serous demilunes at mucous acini, and position of demilunes on mucous parts. Serous and mucous acini were found in the majority of submandibular glands of hamster Nesokia indica, Cricetulus migratorius, Allactaga elater, Funambulus pennati, Meriones libycus and Apodemus sp. However, Jaculus blanfordi contains only serosa acini. Based on the resulted histochemical characteristics, there were remarkable differences among the studied species. In addition, the histochemistry of acini in serosal glands showed that most of the species possess neutral mucin. And there are no mucins in ducts of the major submandibular glands of all species. At two different pH levels, there was weak acidic and sulfated mucin in acini of different species. In addition, laboratory hamster can be differentiated from other species because of the lack of acidic mucin in mucosal acini. There were many differences between convoluted granular tubule and acini in major submandibular glands of all studied species. Convoluted granular tubules in Mus musculus were abundantly present more than serous acini but in Nesokia
indica, laboratory hamster, Cricetulus migratorius, Funambulus pennatii, Meriones libycus, Allactaga elater, Jaculus blanfordi and Apodemus sp., serous acini were higher than convoluted granular tubule. There were also many differences in the types of ducts and types of dominancy of ducts among the studied species. In accessory submandibular glands, the histochemistry of serosa acini, serous demilunes, and the dominancy of mucin based on (sulfomucins sulfate mucins, sialomucins, and neutral mucins) were significantly different among the studied families and subfamilies. Moreover, the dominancy of acini and convoluted tubules and histochemistry of convoluted tubules and serosi acini based on (sulfomucins and acidic mucin and neutral mucins) were different among the studies species (Fig. 1, 2). Therefore, these comparative criteria revealed good inter-species discriminatory potential, the differences can be used very effectively in the comparative inter-species studies, and these differences might be related to factors other than environment and feeding factors. The histological and histochemical characteristics of accessory and major sub mandibular glands showed that these glands are good targets structures in mammalian comparative analysis and should not be ignored by investigators and reflect a good application in veterinary pathology for studying the relatedness of species in regards of certain diseases (3).

References
Effect of Salvianolic Acid B (Sal B) on Osteogenesis of Mouse Mesenchymal Stem Cells

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Keywords: mesenchymal stem cell, osteogenesis, salvianolic acid B,

Introduction

The dried root of Salvia miltiorrhiza Bunge (Danshen) is a popular Traditional Chinese Medicine and has been widely used in both Asian and Western countries for the treatment of various diseases including cerebrovascular diseases, coronary artery diseases, and myocardial infarction. Salvianolic acid B (Sal B; Fig. 1) is the most abundant and bioactive component of salvianolic acid in Danshen. Extensive pharmacological studies have been carried out on this compound. The purpose of this study was to evaluate the cell-biomaterial interaction and the osteogenic capacity of Sal B.

Materials and Methods

The mouse D1 cells were cultured in the presence of osteogenic differentiation (ODM; DMEM with 10% FBS, antibiotics, 50 μg/ml sodium ascorbate, 100 nM dexamethasone, 10 mM β-glycerophosphate) for 6 days, then MSCs were treated to Sal B (3.2~50 μg/ml). Two days later the cells were used for the tests. The cell proliferation was analyzed using MTT assay. Alizarin red staining was done for mineralization. Alkaline phosphatase activity was measured using a commercial ELISA kit.

Results and Discussion

The D1 cells in the culture of ODM differentiated into osteoblasts. The stain by Alizarin red S revealed much higher intensity in ODM cultures with Sal B treatment of 3.2, 6.3, 12.5, 25, 50 μg/ml. The Alizarin red staining had increased with the ODM+Sal B culture (Fig. 2). The cell cytotoxicity of Sal B was not detected (Table 1). The activity of ALP, a marker of osteoblast differentiation, increased after Sal B treatment, as depicted in Table 2.

Salvianolic acid B (Sal B) is the main hydrophilic constituent of Danshen. Previous studies have demonstrated that it has potentiality to improve angiogenesis, osteoblast activity and new bone formation. In this study, we examined the effect of Sal B on osteogenesis of mesenchymal stem cells in vitro. The cell proliferation was analyzed using MTT assay. Alizarin red staining was done for mineralization. Alkaline phosphatase (ALP) activity for cell metabolism was analyzed using a commercial ELISA kit. The mouse MSCs (D1 cells) were converted toward osteoblasts by ODM media culture and Sal B treatment. In the present experiment, ALP activity was significantly stimulated after Sal B treatment (Fig. 2). This phenomenon was supported by the fact that MSCs differentiated into osteoblasts by Sal B.

These data suggest that Sal B enhances the osteogenic differentiation of the mouse mesenchymal stem cells

Acknowledgement

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (KRF-2007-331-E00269).

Table 1. Effect of salvianolic acid B on mouse D1 cell viability

<table>
<thead>
<tr>
<th>Group</th>
<th>Cell viability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMEM</td>
<td>100.0±3.9</td>
</tr>
<tr>
<td>ODM</td>
<td>98.0±2.4</td>
</tr>
<tr>
<td>Sal B 3.2 μg/ml</td>
<td>96.0±3.7</td>
</tr>
<tr>
<td>Sal B 6.3 μg/ml</td>
<td>98.0±2.4</td>
</tr>
<tr>
<td>Sal B 12.5 μg/ml</td>
<td>94.0±2.4</td>
</tr>
<tr>
<td>Sal B 25 μg/ml</td>
<td>96.0±2.5</td>
</tr>
<tr>
<td>Sal B 50 μg/ml</td>
<td>83.0±4.7*</td>
</tr>
</tbody>
</table>

* p<0.0005 as compared with the DMEM control group.

Values are expressed in mean±S.D. (n=3), DMEM: Dulbecco’s Modified Eagle’s Medium (control), ODM: osteogenic differentiation media (DMEM, 50 μg/ml sodium ascorbate, 100 nM dexamethasone, 10 mM β-glycerophosphate).

Table 2. Effect of salvianolic acid B on ALP activity of mouse D1 cell

<table>
<thead>
<tr>
<th>Group</th>
<th>ALP activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMEM</td>
<td>100.0±21.2</td>
</tr>
<tr>
<td>ODM</td>
<td>177.5±20.0</td>
</tr>
<tr>
<td>Sal B 3.2 μg/ml</td>
<td>187.0±23.5</td>
</tr>
<tr>
<td>Sal B 6.3 μg/ml</td>
<td>185.1±20.7</td>
</tr>
<tr>
<td>Sal B 12.5 μg/ml</td>
<td>194.2±53.7</td>
</tr>
<tr>
<td>Sal B 25 μg/ml</td>
<td>255.4±81.5</td>
</tr>
<tr>
<td>Sal B 50 μg/ml</td>
<td>453.0±82.2*</td>
</tr>
</tbody>
</table>

* p<0.05 as compared with the DMEM control group. Values are expressed in mean±S.D. (n=3), DMEM: Dulbecco’s Modified Eagle’s Medium (control), ODM: osteogenic differentiation media (DMEM, 50 μg/ml sodium ascorbate, 100 nM dexamethasone, 10 mM β-glycerophosphate).

Salvianolic acid B (Sal B) is the main hydrophilic constituent of Danshen. Previous studies have demonstrated that it has potentiality to improve angiogenesis, osteoblast activity and new bone formation. In this study, we examined the effect of Sal B on osteogenesis of mesenchymal stem cells in vitro. The cell proliferation was analyzed using MTT assay. Alizarin red staining was done for mineralization. Alkaline phosphatase (ALP) activity for cell metabolism was analyzed using a commercial ELISA kit. The mouse MSCs (D1 cells) were converted toward osteoblasts by ODM media culture and Sal B treatment. In the present experiment, ALP activity was significantly stimulated after Sal B treatment (Fig. 2). This phenomenon was supported by the fact that MSCs differentiated into osteoblasts by Sal B.

These data suggest that Sal B enhances the osteogenic differentiation of the mouse mesenchymal stem cells
Histomoniasis in Broilers: Case Report

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Keyword: broilers, Heterakis, Histomoniasis,

Introduction
Histomoniasis is caused by a protozoan parasite named Histomonas meleagridis. Often called blackhead disease, histomoniasis primarily affects gallinaceous birds (chickens, grouse, partridge, pheasants, quail, turkeys) and also affects to broiler chicken. The clinical signs of infected bird may include lethargy, drooping wings, eyes closed, head held close to the body, weakness, weight loss and sulfur-colored droppings. Morbidity, mortality and culling may reach 20% in chickens (1). Lesions are characterized by thickening and ulceration of the lining of the ceca and by focal necrosis in the liver. The combination of swollen, inflamed ceca with yellow, cheesy cecal cores and discrete spots of necrosis in the liver (yellow-ring necrotizing hepatitis) is considered indicative for histomoniasis. Histomonas meleagridis usually transmitted by the cecal worm Heterakis gallinarum (2). However direct transmission can occur (3). The chickens during 4-6 weeks was prone to infected with Histomonas spp. This report describes the clinical characteristics of histomonas infection of broilers.

Materials and Method
A 2-month old, mixed-breed, broiler flock underwent depression, anorexia, palor and emaciation with approximately 10% mortality. Dead chickens was submitted for diagnosis at Faculty of Veterinary Medicine Kasetsart University, Kamphaengsaen Campus. Upon necropsy, the striking lesions were yellow-ring necrotizing hepatitis and pseudomembranous typhlitis. Organs showing lesions were fixed in 10% buffer formalin and process for routine histopathology.

Results and Discussion
The 3,500 of mixed-breed broilers were reared on semi-intensive system and fed a commercially prepared food. Water is offered at lib. The disease appeared gradually progress with time and most affected broilers die within a period of 1-10 days from appearance of symptoms. Mortem examination showed ulcerative typhlitis with pseudomembrane and focal necrosis in the liver. Lung, kidney and heart were normal. Histopathologically, extensive infection of Histomonas spp., presented by its schizogony in hepatic parenchyma and mononuclear cells infiltration. Typhlitis with necrosis was obvious in ceca. The schizonts and inflammatory cells were present in mucosal, submucosal and muscular layers of the cecum. Although round worm, Heterakis spp. was not found, deworming was done. A pilot study of antiparasitic drug was performed, using dimetridazole at 250 ppm in drinking water for 7 days. The problem disappeared within 2 weeks.

The infection of histomonas in chiken have been previously reported in ducks, tukeys, broiler chickens and game fowl (1) which are reared on ground litter or exposed to range. Histomonas are spread in chicken feces, Heterakis gallinarum (cecal worm) eggs or earth worms. Concurrent infection with cecal coccidias have been reported to aggravate the clinical effect of histomonas infection in broiler chickens (3). In this report, we also found coinfection with cecal coccidias. In conclusion, the present case shows the clinical and histopathological characteristics of histomonas infection of mixed-breed broilers. The significance gross lesions including thyphlitis and yellow-ring necrotizing hepatitis were found. The schizonts of histomonas were presented in hepatic parenchyma and cecum. The diagnosis of histomoniasis was made.

References

Fig. 1-4. The affected mixed-breed broilers were presented with palor, depression and emaciation. (A). Caecum was swollen and inflammed with caecal cores (B). Yellow-ring necrotizing hepatitis was found in liver (C). The schizonts of Histomonas were found in hepatic parenchyma (arrow, D).
Metastatic Malignant Sertoli Cell Tumor with Unilateral Hydronephrosis in a Male Shih Tzu Dog: a Case Report

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Keywords: dog, histopathology, hydronephrosis, malignant, sertoli cell tumor

Introduction

Sertoli cell tumor (SCT) is one of the three most common testicular tumors in dogs (1). It arises from sertoli cell located in seminiferous tubules. The tumor is commonly found in cryptorchid testicle. Breed predilection was reported in schnauzer dog with persistent Mullerian duct syndrome. About 20-30% of dog with sertoli cell tumor exhibited signs of feminization e.g. gynecomastia, male attractive, pendulous penile sheath, atrophy of contralateral testis, bilateral symmetrical alopecia and epidermal atrophy (2). Most sertoli cell tumors are benign. Metastatic rate is very low for tumor smaller than 2 cm. in size. Larger tumors are prone to metastasize especially to lymph nodes of sublumbar and pelvic regions. Two histological forms including intratubular and diffuse forms were categorized (3).

The aims of this study are to present gross and microscopic features of a dog with metastatic malignant sertoli cell tumor and to report a possible consequence of this tumor in dog.

Materials and Methods

Case History: A 7-year-old male Shih Tzu dog with signs of severe non regenerative anemia and right side inguinal cryptorchidism was clinically investigated. Clinical and ultrasonographic examination revealed a large mass within abdominal cavity. Laparoscopy found a large tumor mass with large flaccid right kidney and moderate amount of red effusion which was further classified as hemorrhagic transudate. The tentative diagnosis of sertoli cell tumor with large abscess at right kidney was made. The animal died few days later due to internal bleeding and shock. The carcass was submitted for necropsy at Mahanakorn Veterinary Diagnostic Center (MVDC). Necropsy was done and the samples were collected for histopathology using conventional method. Briefly, the tissues were processed and embedded in paraffin, sectioned on 4 micron and stained with hematoxylin and eosin (H&E). All slides were examined under light microscope. Immuno-histo-chemical staining was performed on paraffin sections using standard protocol of labeled streptavidin-biotin technique (LSAB). Antibody against vimentin (1:1000 V9, Neomarker, USA), pancytokeratin (1:200 AE1/AE3, Neomarker, USA) and Ki-67 (1:50 SP6, Neomarker, USA) were applied. The results were observed under light microscope.

Results and Discussion

Necropsy result: General appearance, the skin showed bilateral symmetrical alopecia with enlargement of all nipples and pendulous prepuce. Within abdominal cavity, a large multi-lobulated, encapsulated mass of 12x10x12 cm. was found at right lumbar area adjacent to the caudal pole of right kidney and entrapped upper part of right ureter. The urine outflow from affected kidney was prohibited and severe hydronephrosis was the consequence (Fig.1). Right testis was found at inguinal area (congenital cryptorchidism) without direct connection to abdominal mass. A tumor mass of 2.5x2x2.5 cm. with necrosis and hemorrhages were detected in right testis (Fig. 2) and epididymis (1.5x1x1.5 cm.). The contralateral testis was atrophic. Other organs including organs of alimentary system, respiratory system, nervous system, hemo-lymphopoietic system, adrenal gland and heart remained intact.

Histopathological result: The large well demarcated, encapsulated intra-abdominal mass, mass at right testis and epididymis composed the same type of neoplastic cells. Within the mass, multiple cords or sheath of neoplastic elongated cells were found with fibro-vascular stroma. The tumor cells arranged perpendicularly to the stromal scaffold (Fig. 3). Most cells had narrow cytoplasm often vacuolated. The nuclei were mostly oval with less heterochromatin and prominence one or two concentric nucleoli. Mitotic figures ranged from 0-3/HPF with few atypical mitoses (Fig. 4). Anisocytosis and anisokaryosis were observed (Fig. 5). Areas of necrosis and hemorrhages were present in especially in large tumor (Fig. 6). The contralateral testis revealed completely atrophic (Fig. 7). The skin showed severely epidermal atrophy, less number of hair follicles with hyperkeratosis of hair follicles, which mostly in telogen phase.
**Immunohistochemistry result**: The tumor cells stained positively for vimentin (Fig. 8) and negatively for pancytokeratin. This confirmed mesenchymal origin of tumor cell. Rare tumor cells showed immune reactivity to Ki-67, proliferation marker.

**Morphological diagnosis**: Malignant sertoli cell tumor at right cryptorchid testis with metastasis to epididymis and sublumbar lymph nodes and subsequence hydronephrosis in right kidney.

Cryptorchidism was observed about 10% of male dogs. The occurrence in right testis was two times more than the left possibly due to longer distance to descent into scrotal sac (1, 7-8). In our case, inguinal cryptorchidism was also found in right testis and sertoli cell tumor was detected without significant enlargement of the testis. Gross and histopathological findings of necrosis and hemorrhage within the tumor mass may explain why the affected testicle showed no remarkable enlargement. The larger size of metastatic tumor in sublumbar lymph nodes and severe hydronephrosis with completely loss of right renal tissue indicated the longstanding period of occurrence. The pathogenesis of hydronephrosis is clearly due to compression of enlarged sublumbar metastatic tumor nodule on right ureter adjacent to renal pelvis. In the literature, metastasis was detected in approximately 10% of SCT cases. The routes of metastasis were reported via lymphatic route to inguinal and sublumbar lymph nodes. Distance metastasis was found in liver, lung, spleen, kidney and adrenal gland (2). Hydronephrosis as a consequence of metastatic malignant sertoli cell tumor as found in this case has never been reported before. Atrophy of contralateral testis mentioned in the literature was due to inhibitory effect by excessive estrogen on anterior pituitary gland to secrete gonadotrophic hormone (3). This was also found in this animal. The dog in this case showed signs of hyperestrogenism e.g. swelling and pendulous prepuce, enlarged and elongated nipples, atrophy of epidermis and bilateral symmetrical alopecia. In the literature, feminizing syndrome was reported in one third of SCT cases in dogs (4). Additionally, the effect of prolong hyperestrogenism on bone marrow may result in bone marrow hypoplasia with subsequence non-regenerative anemia as also observed in this animal.

This report may remind veterinary surgeons or the owners about the effect and outcome of delayed castration after the first detection of the cryptorchid testicle.

**Acknowledgements**

The Author thanks Mahanakorn University of Technology (MUT) for financial support of this study. Special thanks were also given to Staff of Small Animal Teaching Hospital for clinical information and to Dr. Hassadin, Dr. Suwarin and Ms. Siriwan, staff of MVDC, for all technical supports.

**References**

Localization of Prostaglandin E2 Receptor Subtype 4 (EP4) in the Cervical Tissue of Bitches Developing Pyometra

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Keywords: dog, EP4, prostaglandinE2, pyometra

Introduction
Pyometra is a reproductive disease occurring in one fourth of female dogs over 10 years of age (1). The most effective treatment is ovariohysterectomy (OVH) because this surgical technique prevents recurrence of the disease. However, surgery has its limitation when bitches are at risk of anesthesia and in life threatening. The alternative treatment is medical therapy such as prostaglandin F2α given to expulse exudates from the uterus but its side effect especially uterine rupture can occur in the cases of closed-cervix pyometra (2). Current literatures in other mammals indicate that PGE₂ play an important role in cervical ripening (3). PGE₂ exerts its roles by coupling to prostaglandin E₂ receptors (EP) of four subtypes (EP1, EP2, EP3 and EP4) (4). In cervical tissue of pregnant rats, the expression of EP4 at term of labor is much higher than during early pregnancy and estrous stage (5). PGE₂ has been used as cervical ripening agent in human (6) and mares (7) but has not been studied in dogs. The aim of this study was to investigate the localization of EP4 and its regulation on cervical patency in pyometra bitches.

Material and Methods

Immunohistochemistry: Cervical tissues of bitches with open- (n=6) and closed-cervix pyometra (n=6) were obtained after ovariohysterectomy at the Small Animal Teaching Hospital, Chulalongkorn University. The tissues were fixed in 4% paraformaldehyde, embedded in paraffin blocks, cut into 4-μm sections and mounted on silane-coated slides. The immunohistochemical detection of EP4 was performed by using goat polyclonal anti-human EP4 (C-18: sc-16022, Santa Cruz biotechnology, CA, USA) in humidified chamber. The avidin-biotin method was used as described in ABC elite kit (Vectastain® ABC kit, Vector Lab., CA, USA). The complex was visualized with NovaRed (Vector Lab., CA, USA). Sections were counterstained with Mayer’s hematoxylin and finally mounted in glycerine gelatin.

Quantification for immunohistochemistry: The analytical processes of expression were performed semi-quantitatively in 4 different tissue layers (i.e. surface epithelium, subepithelium, glandular epithelium and myometrium. The intensity of the staining was divided into 3 grades which were weak, moderate and strong staining. The proportion of each intensity score was rated and calculated to average value. The expression index was calculated from the percentage expression multiplied by the average intensity score of staining.

Statistical analysis: Means±S.E.M. of expression index were presented. Mixed model analysis of variance (SAS version 9.0) was used to compare differences of the expression index between layers (SE-surface epithelium, Sub-subepithelium and Mus-muscular layer) of each group. Significant difference was set as p<0.05.

Results and Discussion
Immunohistochemical staining demonstrated that EP4 expressed in all layers of canine cervical tissue of open- and closed-cervix pyometra (Fig. 1). Although the expression was found in all layers, the highest intensity
of positively stained cells was found in the Mus and lowest in the Sub (Table 1). The mean (±S.E.M) of expression index in the SE was higher in the open-(67.1±15.9) than closed cervix pyometra (39.2±11.4) \( (p<0.05) \). However, the differences of the expression in SE and Mus were not observed between groups \( (p<0.05) \).

**Table 1** Mean±S.E.M. of expression index of EP4 in cervical tissues of bitches developing pyometra.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>SE</th>
<th>Sub</th>
<th>Mus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opened cervix pyometra</td>
<td>6</td>
<td>67.1±15.9(^a)</td>
<td>84.5±13.0(^a)</td>
<td>109.2±8.7(^a)</td>
</tr>
<tr>
<td>Closed cervix pyometra</td>
<td>6</td>
<td>39.2±11.4(^a)</td>
<td>63.9±12.6(^a)</td>
<td>96.4±12.9(^a)</td>
</tr>
</tbody>
</table>

EP4 expression in the surface epithelium was higher in open-cervix pyometra than closed-cervix pyometra, suggesting that PGE\(_2\) might involve in cervical dilatation by acting through EP4. The previous study revealed that EP4 induces cervical dilatation via relaxation of the smooth muscle (8). Furthermore, the expression of EP4 was greatest in Mus, demonstrating that activation of EP4 likely to cause relaxation of smooth muscle as shown in the previous study (9). Therefore, the expression of different subtypes of prostaglandin receptors (EP1, EP2, EP3 and EP4) in the bitch cervix may be value further studied.

**Acknowledgements**

This research was funded by faculty of Veterinary Science, Chulalongkorn University.

**References**

Effects of Hot and Humid Climates on the Number of Mummified Fetuses in Gilts and Sows

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Keywords: pig, reproductive performance, Temperature-humidity index, tropics

Introduction
During the last decade, global warming has become a major concern for humans. Official data from the meteorological department in Thailand indicate that the environmental temperature increased during the period from 1996 to 2005 and has tended to continue to increase. The increase of the environmental temperature also has a potentially large impact on the pig industry, especially for pigs that are housed in a conventional open-housing system, which is the most common type of housing of swine commercial herds in Thailand. It is well-established that high ambient temperature and high humidity as well as a tropical climate negatively influence the reproductive performance of female pigs (1-5). A common feature of the seasonal influence on the gilt’s and sow’s reproductive performance include prolonged weaning-to-first-service interval, decreased conception and farrowing rates, increased remating rate, and increased embryonic loss (1-5). To our knowledge, no comprehensive study on the influence of climatic factors, e.g., temperature, humidity and temperature-humidity index (THI) on the number of fetal loss in pig under tropical climate has been done. The aim of the present study was to use data from herds to demonstrate the influence of THI on the proportion of mummified fetuses per litter in gilts compared to sows parities 2, 3-5 and ≥6 in a conventional, open-housing system for swine commercial herds in the northeastern part of Thailand.

Materials and Methods

Data: Data were obtained from four swine commercial herds (A, B, C and D) in the northeastern part of Thailand. The data included sows farrowed during the period from July 2005 to June 2008. The herd data were obtained from the computer recording system of the herds from January 2005 to December 2008. The data included the sow’s identities, farrowing date, parity number, number of piglets born alive per litter (BA), number of stillborn piglets per litter (stillborn), number of mummified fetuses per litter (mummy), litter’s birth weight, piglet’s birth weight and number of piglets at weaning. The total number of piglets born per litter (TB) was calculated by summing of BA, stillborn, and mummy totals. The proportion of mummified fetuses per litter (MF) was calculated using the number of mummified fetuses divided by TB and multiplied by 100. The data included observations on 25,835 litters from 8,100 sows.

Herd location and management: The four herds in the present study were located in the northeastern part of Thailand between latitude 14-17°N and longitude 102-103°E. The housing facilities in Herds (herd size) A, B, C and D were available for 1,200, 1,500, 1,000 and 500 sow inventories, respectively. The breeds of the sows were predominantly crossbred LY, and were mainly bred with Duroc or hybrid boars (PIC® Siam Ltd., Thailand). Conventional artificial insemination (AI) was used for all gilts and sows. In all herds, gilts and sows 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. In general, the gilts were mated at ≥32 weeks of age with a BW of ≥135 kg at the second or later observed oestrus. The health of the herds was monitored by the herd veterinarians. In general, the veterinarians gave the recommendation to vaccinate the gilts/sows against foot-and-mouth disease (FMD), swine fever (SF), Aujeszky’s Disease (AD), porcine parvo virus (PPV) and arthropic rhinitis (AR), at between 22-30 weeks of age in replacement gilts, and during late gestation (FMD, AR) and during lactation (PPV, SF) in sows. Mass vaccination of AD was conducted every four months. All herds were porcine reproductive and respiratory syndrome (PRRS) sero-positive herds, but no clinical outbreak was observed during the period of study. Culling due to old age was planned to be done after parity six. 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. All of the herds were visited monthly by the first author of this study to monitor routine management and health.

Meteorological data: Outdoor temperature and humidity data were obtained from July 2005 to June 2008 from an official
meteorological station within 100 km from the herds. The average minimum-maximum daily temperatures were 21.1-33.3°C, 24.4-31.6°C and 17.9-29.9°C in the hot, rainy and cool seasons, respectively. The 24-h average humidity was 68.3%, 81.7% and 64.2% in the hot, rainy and cool seasons, respectively. THI was calculated using the following formula (6): 

\[
\text{THI} = DB - (0.55 - (0.55 \times RH) \times (DB - 58))
\]

where DB is the average daily temperature and RH is the average daily humidity. On average, the THI was 79.2±3.5, 79.7±1.7 and 73.5±4.2 in hot, rainy and cool seasons, respectively.

Statistical analyses: The statistical analyses were carried out using SAS (SAS 2002). The influences of THI on MF were analyzed using the general linear mixed model procedure of SAS. The meteorological data were merged with the reproductive data by farrowing date (farrowing year-month-day). The means of THI during 115 days before farrowing were calculated and were used in the statistical models. The statistical models included herds (A, B, C, D), parity groups (1, 2, 3-5, 6-12), years (1, 2, 3), THI classes (71-72, 73-78, 79-80 and ≥81), and two ways interactions between parity and THI and between herd and THI. Since the sows included in the analyses produced 3.2±1.8 litters/sow (range 1-8 litters/sow) during the study period, the sow ID was included in the statistical model as a random effect. The classification of THI was based on information from earlier studies (7) and the frequency of the THI data. 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, the gilts and sows in commercial herds in Thailand kept in the open-house system had 11.3±2.9 TB, 10.2±2.9 BA, 2.2% MF, 7.3% SB and 9.5±1.9 piglets at weaning. The means THI during gestation periods influenced MF (\(p=0.06\)). MF varied among herds from 1.6% in herd C to 2.9% in herd B (\(p<0.001\)). On average, MF was 3.3%, 1.8%, 1.9% and 2.6% in parity 1, 2, 3-5 and 6-12, respectively (\(p<0.001\)). The influence of the THI during gestation period on MF by parity groups are demonstrated in Fig. 1. As can be seen from the figure, the influence of the THI was more pronounced in the 1st parity than parity 2, 3-5 and 6-12.

It is known that a pig regulate internal temperature within a narrow range by matching the amount of heat produced through metabolism with the heat flow from animal to the surrounding environment. Under field condition, hyperthermia often occurs in pig due to the heat flow from the animal is less than internal heat production (7). In beef cow, it has been shown that increasing environmental temperature and relative humidity from 21°C, 35% relative humidity to 37°C, 38% relative humidity, during days 8th to 16th of gestation period reduced the size and weight of the conceptus (8). In Brazil, Bényei et al. (7) demonstrated that the average number of corpus luteum of superovulation cows decreased from 9.8 to 5.2 when THI increase from 70.7 during cool season to 79.7 during the El-Nino phenomenon. Earlier studies demonstrated that the high ambient temperature not only influence early embryonic and/or fetal loss, but also influence the follicular development and hence decrease the number of ovulation, the oocyte quality and/or fertilization rate (7-9).

In the present study, the average THI was 79.2±3.5, 79.7±1.7 and 73.5±4.2 in hot, rainy and cool seasons, respectively. The average THI observed during hot and rainy seasons in Thailand are almost equal to the THI that observed during the period of El-Nino phenomenon in Brazil (79.7±4.0) (7). This level of THI significantly reduced the number of ovulation and the oocytes quality in cows (7). The present study revealed that THI above 81 significantly increased MF in primiparous sows, but not in multiparous sows. Therefore, the control of environmental temperature and humidity for pregnant gilts should be emphasized.

Acknowledgements

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References

Introduction

Porcine reproductive and respiratory syndrome (PRRS) is caused by an envelope, single-stranded positive-sense RNA virus known as PRRSV (PRRSV) (1). The disease was observed first time in the United States of America (USA) since the late 1980s (2) and was found in Europe since 1990 (3). In Thailand, PRRSV sero-positive pig has been observed as early as 1989 (4). In 1995, the seroprevalence of PRRS was, on average, 64% with a variation among the herds from 20% to 90% (5). In general, PRRSV is classified according to their genotype as North American (US) and European (EU) strains (1, 4). In Thailand, both US and EU strains have been isolated (4). PRRSV causes many signs of reproductive failure in gilts and sow, such as infertility, abortion, death of sows and pre-weaning mortality (1, 6). Under field conditions, the infected sows develop a protective immunity and usually produce normal litters after rebreeding although the virus still circulate within the herds (7). The duration of the protective immunity is in fact unknown, but at least 604 days post infections have been proposed (7). Larger et al. (8) demonstrated that homologous PRRSV protective immunity was produced within 90 days post exposure and the virus-specific antibody was detected for at least 110 days post exposure in adult pig. Up to date, intensive acclimatization and/or vaccination in replacement gilts are commonly practiced in most breeding herds. However, high variability of the antibody titer against PRRS of the gilts is still observed both within and between herds. The objective of the present study was to retrospectively investigate the seroprevalence of PRRSV antibody in pigs in 5 commercial herds during 2004-2007. Furthermore, the seroprevalence of PRRSV between herds that vaccinated the gilts and sows with modified live virus (MLV) vaccine and those that performed intensive acclimatization in replacement gilts were compared.

Materials and Methods

Animals and data: The study was conducted in 5 commercial swine herds (A, B, C, D and E) in Thailand during January 2004 to December 2007. A total of 5,664 blood samples from 544 boars, 1,164 sows, 3,168 replacement gilts, 486 nursery pigs and 302 fattener pigs were collected and determined for PRRSV-specific antibody titer.

Herd location, management and vaccination: The herds in the present study are located in the eastern (A, E), middle (B), western (C) and northeastern (D) of Thailand between latitude 13° and 17°N and between longitude 100° and 104°E. All herds included in the present study were breeding herd and the sows on production numbering about 900-3,500 sows/herd. Two herds (A and D) produced the replacement gilts within the herds, while 3 herds (B, C, E) bought the replacement gilts from other breeders. In general, the gilts entered the gilt pools at about 22-24 wk of age at 80-100 kg body weight (BW). Water was provided to ad lib from water nipples. The feed were provided twice a day (about 3 kg/day). The gilts were kept in a pen with a group size of between 6-15 gilts/pen with a space allowance of 1.5-2.0 m2/gilt and pregnant gilts and sows were kept in individual stall. Lactating sows were kept in individual pens. In most cases, the gilts breed the replacement at ≥32 week of age with a BW of ≥130 kg at the second or later observed oestrus. Boar contact and estrous detection was applied to the gilts between 24-35 wk of age. The health of the herds was controlled by the herd veterinarian. In all herds, removal sows were taken to acclimatize the gilts for about 4 weeks period with a ratio of 1 sow per 6-10 gilts. The acclimatized sows were rotated weekly. Before breeding, the gilts were vaccinated against Foot-and-mouth disease (FMD), Swine fever (SF), Aujeszky’s disease (AD) and Porcine Parvo virus (PPV) vaccine during 22-30 week of age. In herd B, the gilts, sows and nursery pigs were also vaccinated against US-strain of MLV vaccine (Ingelvac® PRRSTM MLV, Boehringer-Ingelheim Vetmedica Inc., Missouri, USA), and in herd C and E, the EU-strain of MLV vaccine (AMERVAC®, Lab. Hipra, Spain) were used.

Serological test: Antibody of PRRS virus was tested by using HerdCheck PRRS virus antibody test kit 2XR® (IDEXX Lab., Inc., USA) (herd A, B, C and D). Briefly, the positive and negative control was also carried in the same plate as the sample. 100 μl of serum samples was added to the testing plate that coated with PRRS antigen and to the normal host cell (NHC) and incubated at room temperature for 30 min. Anti-Porcine: HRP conjugate was added into the plate 100 μl for each sample and incubated. 100 μl of TMB substrate was added and incubated and then 100 μl of stop solution was added. OD was measured using ELISA reader at 650 nm. The serum sample/positive control (S/P) was calculated. The S/P ratio below 0.4 indicated that the sample had no antibody of PRRSV (negative), while S/P ratio ≥ 0.4 indicated that the sample had antibody of PRRSV (positive).

Statistical analyses: The statistical analyzed was performed using SAS (SAS version 9.0, Cary NC, USA.). Frequency analysis was conducted using PROC FREQ of SAS. The proportional data were analyzed using Chi-squared test, p<0.05 were regarded to be statistical significance.
Results and Discussion

Of all 5,664 tested samples, 4,492 pigs (79.3%) had antibody titer against PRRSV. The proportion of PRRS positive pigs were 79.4%, 82.0%, 82.6%, 84.1% and 48.4% in boars, sows, gilts, fattener and nursery pigs, respectively (Figure 1). The proportion of PRRS positive pigs were 81.7%, 67.9%, 60.6%, 80.9% and 79.3% in herds A, B, C, D and E, respectively \((p<0.001)\). The S/P ratios were 1.5±1.1 (range 0-4.5), 1.3±1.2 (range 0-4.9), 1.0±0.9 (range 0-3.7), 1.4±0.9 (range 0-4.3) in herds A, B, C and D, respectively. Across the herds, the proportion of PRRS negative pigs varied among years from 36.6% in 2004 to 25.6%, 15.7% and 11.3% in 2005, 2006 and 2007, respectively \((p<0.001)\). The proportion of PRRS positive boars varied among years from 69.2% in 2004 to 80.0%, 74.1% and 83.3% in 2005, 2006 and 2007, respectively \((p=0.03)\). In the fattener, the proportion of PRRS positive pigs varied from 82.3% to 89.1% among years \((p=0.7)\). The proportion of PRRS positive gilts were 62.1%, 78.6%, 91.5% and 94.4% in 2004, 2005, 2006 and 2007, respectively \((p<0.001)\). The proportion of PRRS positive nursery pigs were 58.2%, 40.8%, 46.5% and 55.0% in 2004, 2005, 2006 and 2007, respectively \((p=0.03)\). Proportion of PRRS positive pigs from 2004 to 2007 in the MLV PRRSV-vaccinated and non-vaccinated herds are demonstrated in Figure 2. Comparing between the PRRSV vaccinated and non-vaccinated herds, the proportion of PRRS positive pig was demonstrated in Figure 2. A higher proportion of PRRS-specific antibodies fattener pig was observed in the PRRSV-non-vaccinated herds than the PRRSV-vaccinated herds \((p<0.05)\) (Fig. 3).

The present study provided descriptive data on the prevalence of PRRSV infection in 5 swine commercial herds in Thailand. The data indicated that the proportion of pigs infected with PRRS differed among herds, years and groups of pigs. The infection was found to be highest in the fattener (84.1%) and lowest in the nursery pigs (48.3%). High proportion of PRRS positive pigs were also observed in replacement gilts (82.6%) and sows (82.0%). Surprisingly, a relatively high prevalence of PRRS was found in the boars (79.4%). These indicate that the PRRSV circulation and re-infection remain relatively high either in vaccinated or in non-vaccinated herds. Boars, sows and replacement gilts seem to be the important reservoir of the virus. Interestingly, the exposure of PRRSV in the fattener pigs tended to be lower the vaccinated than the non-vaccinated herds.

Acknowledgement

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References

Porcine Reproductive and Respiratory Syndrome Virus Antigen Detection in the Uterine Tissue of Gilts Correlated to the Antibody Titer

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Keywords: immunohistochemistry, pig, PRRS, reproduction, uterus

Introduction

Porcine reproductive and respiratory syndrome (PRRS) is caused by PRRS virus (PRRSV), a member of Arterivirus, family Arteriviridae (1). In general, the infection of PRRS in gilts and sows is characterized by late term abortion, mummified fetuses, stillborn piglets and low viability piglets at birth (2-4). The antibody titers against PRRSV infection are detected by 7-14 days after the animals are infected and remain for several months before declining (5). Under farm condition, intensive acclimatization and/or vaccination in replacement gilts are commonly practiced in most breeding herds. However, high variability of the antibody titer against PRRSV of the gilts is observed both within and between herds. This problem causes difficulties for the farmer to make decision to mate the gilts. Additional knowledge concerning the antibody titer of PRRS in the replacement gilts in different herds is needed to be investigated. It has been suggested that replacement management of gilts is a major source of introducing new strains of PRRSV into the herd. Our previous study has found that 73% (122/166) of the replacement gilts culled due to reproductive disturbance had been infected with PRRSV. In addition, a higher proportion of seropositive gilts was particularly found in those that were culled due to abortion (81%) and repeat breeding (81%) (6). It is well established that alveolar macrophages as well as macrophages from other tissues are the primary cell type sustaining the in vivo replication of the viruses (7). Using Immunohistochemistry (IHC) for evaluating formalin-fixed tissues, it was found that 66% and 100% of the lung tissue of piglets infected with US and EU strains of PRRS have been observed, respectively (7). An earlier study has demonstrated that 75.0%, 50.0%, 37.5%, 37.5%, 37.5% and 25.0% of IHC positive cells was observed in liver, spleen, tonsil, turbinate bone, pulmonary lymph node and ileum of the infected piglets, respectively (7). To our knowledge, the expression of PRRSV in the uterine tissue of gilts has not been demonstrated. The objective of the present study was to determine the incidence of PRRSV in the uterine tissue of gilts in relation to the level of antibody titers.

Materials and Methods

Uterine tissues from 50 replacement gilts were collected from three commercial swine herds (A, B and C) in Thailand. All of the gilts were culled due to reproductive disturbance. The culling reasons included anestrus (n=29), vaginal discharge (n=10), repeat breeding (n=5), abortion (n=5) and not being pregnant (n=1). Historical data for all gilts was collected. All herds included in the present study were breeding herds and the sows on production were between 900-3,500 sows/herd. Herd A produced replacement gilts within the herd using their own grand parent (GP) stock, while herds B and C bought the replacement gilts from other breeders. The gilts in all herds were housed in a conventional open housing system facilitated with a water sprinkler and fan. The health of the herds was monitored by the herd veterinarian. In general, the veterinarian gave the recommendation to vaccinate the gilts against foot-and-mouth disease, classical swine fever, Aujeszkyís disease and porcine parvovirus (PPV) at between 22-30 weeks of age. In addition, herd B vaccinated the replacement gilts using US-strain modified-live virus (MLV) vaccine (Ingelvac® PRRSTM MLV, Boehringer-Ingelheim Vetmedica Inc., St. Joseph, Missouri, USA), while herd A and C vaccinated the gilts using EU-strain MLV vaccine (AMERVAC®, Lab. Hipra, Girona, Spain). Blood samples were collected from jugular vein of the gilts prior to culling. Serum were obtained and kept at -20°C for analyzing antibody titer of PRRSV. After slaughter, the ovary and uterus were collected, placed on ice and transported to the laboratory within 24 h of culling. Tissue samples were collected from the uterus of the gilts, fixed in 10% neutral buffered formalin for at least 24 h and embedded in paraffin blocks. Immunohistochemistry (IHC) was performed on the uterine tissues of the gilts using the protocol of the lung tissue with some modification (7). A polymer-based non-avidin-biotin technique was applied in the present study. Primary monoclonal antibody SDOW17 (Rural Tech., Inc., USA) diluted 1:1000 was used. Negative control procedures included omission of primary antibody. Known PRRSV-positive lung and lymph node tissues served as positive controls. The sections were interpreted as positive if contained at least 1 positive cell (brown intracytoplasmic staining, Fig. 1). PRRSV antibody was
determined using a commercial enzyme-linked immunosorbent assay test kit (ELISA, HerdChek® PRRS virus antibody test kit 2XR, IDEXX Lab., Inc., USA). The protocol followed the kit’s instructions. The serum sample/positive control (S/P) was calculated. The S/P ratio below 0.4 indicated that the sample had no PRRSV antibody (negative), while S/P ratio ≥ 0.4 indicated that the sample had PRRSV antibody (positive). Statistical analyses were performed using SAS (SAS, 2002). The percentage of positive tissue was compared with the detection of antibody titers against PRRSV by using ELISA (positive and negative) using Fisher’s exact test. \( p < 0.05 \) was considered as statistically significant.

**Results and Discussion**

PRRSV antigens were detected in the cytoplasm of macrophage-like cells in the sub-epithelial connective tissue layers of the endometrium in 28% (14/50) of the gilts. The PRRSV positive cells were observed in the cytoplasm of the macrophages in the endometrium (Figure 1). Of all the gilts, 77.6% (38/49 gilts) were positive to the ELISA test (Table 1). Of the seropositive gilts, 28.9% (11/38) had PRRSV antigen in the uterine tissues, while 18.2% (2/11) of the seronegative gilts had PRRSV antigen in the uterine tissues (\( p = 0.70 \)). Compared to the seronegative gilts, seropositive gilts had a 1.83 (95% confidence interval=0.34-9.89) higher odds for detecting PRRSV antigen in the uterine tissue. Among the seropositive gilts, high level of antibody titer (S/P ratio ≥1.2) was found in 47.4% of the gilts. The incidence of IHC-PRRS positive staining cells was found in 33.3% of the high antibody titer gilts and in 25.0% of low antibody titer gilts (\( p = 0.72 \)).

The present study demonstrated the present of PRRSV in the uterine tissue of gilts. The site of positive cells was at the subepithelial layer of endometrium. PRRSV infection is a multisystemic disease which is characterized by viremia and subsequent virus distribution and replication in multiple organs (8). In the present study, it was found that gilts that had PRRSV antigen in the uterine tissue were culled at 287 days of age. Most of these gilts have been sent into the breeding herd and might shed the virus to the susceptible pigs in the herd. In the present study, the percentage of gilts culled due to reproductive disturbance that were detected the antibody against PRRSV was in agreement with the earlier study (6). It was not surprise to see both seropositive and seronegative gilts had PRRSV antigen presented in the uterine tissue of the culled gilts since PRRSV antibody titer cannot determine the persistent infection. Although the proportion of IHC-PRRS positive gilts tended to be higher in the gilts that had a high level of S/P ratio, a certain amount of the IHC-PRRS positive uterine tissue were also observed in the gilts with low S/P ratio and even in the PRRSV seronegative gilts. This imply that the use of antibody titer as a criteria to introduce replacement gilts into the breeding house may not be good enough and remain a risk of introducing IHC-PRRS positive gilts into the herds. It has been demonstrated that the duration of protective immunity against homologous strain of PRRSV may persist for at least 604 days post experimental exposure to the field PRRSV, while the duration of detectable PRRSV-specific antibodies that develop in sows following natural infection is thought to be as short as 4-8 months (9). These findings suggested that replacement gilts must be allowed to expose homologous strain of PRRSV before entering the breeding herds.

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**References**

Malignant Paraganglioma Case in a Siberian Tiger

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Keywords: chromogranin A, paraganglioma, siberian tiger, synaptophysin

Introduction

Paraganglioma is rare tumors originating in cells of neural crest origin in the extra-adrenal paraganglia of the autonomic nervous system (1, 2). It occurred mainly in the abdominal cavity, head and neck (3). In this report, we present histopathologic and immunohistochemical evidence of malignant paraganglioma originated from retroperitoneum of a Siberian tiger, with subsequent systemic metastasis.

Material and Methods

A 14-year-old female Siberian tiger with anorexia and depression was dead. From pathologic examinations, paraganglioma was tentatively diagnosed. For more definitive diagnosis, we performed immunohistochemistry against synaptophysin (1: 20, abcam), S100 (1: 50, Dako), cytokeratin (1: 10, 34/E12, Dako), pan cytokeratin (1: 5, AE1/AE3, abcam), Gila fibrillary acidic protein (GFAP) (1: 250, Dako) chromogranin A (CgA) (1: 1600, Enzo) and vimentin (1: 200, Dako).

Results and Discussion

Grossly, several masses were observed throughout the body including 6x3x3 cm sized oval mass in the retroperitoneal cavity, a 10x5 cm mass attached ventrally to lumbar vertebrae and a 4x4x3 cm spherical mass attached to parietal pleura ventral to the heart. The peritoneal masses were firm and encapsulated with fibrous tissues with well developed blood vessels. Histologically, the tumor cells were arranged in lobular structure surrounded by delicate fibrovascular stroma. The cells had distinct borders, granular eosinophilic plump cytoplasm and binucleate or karyomegalic nuclei. Furthermore, metastasis occurred in the uterus, kidney, adrenal gland, lung and thymus. In immunohistochemistry, the tumor cells are negative for anti-S100, cytokeratin, pan-cytokeratin, vimentin, GFAP. Synaptophysin was intensely stained in the cytoplasm of the tumor cells. Malignant paraganglioma was made as a final diagnosis.

Paragangliomas are rare neuroendocrine neoplasms. It is highly vascular and usually characterized by slow expansion and rarely metastasis. Immunohistochemical and histopathological features are used in the diagnosis of this tumor.

In this case, negative staining of the tumor cells for cytokeratins and vimentin indicates that this tumor is not originated from epithelia and/or mesenchymes. The diffuse staining for synaptophysin and partial staining for CgA mean that the origin of this tumor is neural neuroendocrine cells (4). We thought that negative staining for S100 and GFAP is due to nonspecificity of the antibodies. This case would have a great value to report as a rare paraganglioma case being occurred in the tiger.

References

Peroxidative Injury of Rats Intratracheally Instilled with Benzo(a)Pyrene and Benzo(e)Pyrene

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Keywords: intratracheally, MDA, oxidative stress, PAHs.

Introduction
An increasing amount of research has focused on the role of oxidant/antioxidant imbalance occurs in lung pathologies due to exposure to polycyclic aromatic hydrocarbons (PAHs), the main constituent of air pollutants (1). The inert PAHs, (benzo(a)pyrene (BaP) and benzo(e)pyrene(BeP)), are metabolized locally in the lung epithelia (2) leading to the generation of harmful intermediates and reactive oxygen species (ROS) released by the inflammatory leukocytes, both neutrophils and macrophages (3). These ROS degrade polyunsaturated lipid, particularly of the cell membrane, forming malondialdehyde (MDA) (4). The traditional role for MDA is reacting with deoxyadenosine and deoxyguanosine in DNA, forming DNA adducts, which is mutagenic (5). The guanidine group of arginine residue condenses with MDA to give 2-aminopyrimidines, causing peroxidative injury through increased oxidative burden (6). The production of this aldehyde is used as a biomarker to measure the level of oxidative stress in an organism (7). The aim of this pulmonary study is spatial assessments of MDA production and to determine the sustainability of any observed effect in PAH exposure.

Material and Methods
All animal experiments and animal care were performed according to the Guides for the Care and Use of Laboratory Animals as approved by the Animal Care And Use Committee of the Universiti Putra Malaysia-Faculty of Veterinary Medicine (AUP No: 08R28/July 2008). Ninety, 6-8 weeks old, 150-180 gm, male Sprague Dawley rats, purchased from Northern RK company, were used for the present study. They were housed in separate cages comprising 2-3 rats each and kept in an air-conditioned room (23±1°C, 50-60% humidity) and 12 h light per day. The rats were fed with standard rat pellet and drinking water was made available ad libitum. A single dose (0.1 ml/kg bw) of long acting 20% oxytetracycline HCl was given intramuscularly as prophylaxis.

Animals were divided into 3 equal groups after a 1-week of adaptation period. Rats receiving only tricaprylin acted as controls. Cumulative individual doses of 13.8 ng and 13.0 ng of BaP and BeP, respectively calculated on its occurrence in Malaysia haze 1997 (8), were i.t. administered (9) at 8 frequencies for a month to the BaP and BeP groups.

Blood (2 ml) was collected by cardiac puncture into hepanirised tubes before treatment (day 0), hours (12 and 32), days (3, 7, 21, 60 and 180) p.i for estimation of MDA. Five rats from each group were necropsied at 12 hours, 3, 7, 21, 60 and 180 days p.i, after an i.m injection of ketamine:xylazine. Lung and liver samples procured at post mortem were snap frozen in liquid nitrogen and kept at (-80°C) until further use. Homogenates of lung and liver were prepared by mixing it with cold 1.15% KCl (1:10, w/v). The homogenates was centrifuged at 10,000g for 20 min and the supernatant was used for MDA assay (10).

The data were analyzed with SPSS 16.0 for Windows (SPSS Inc., Chicago, IL) by using one-way analyses of variance (ANOVA). Differences between means were determined using Tukey’s multiple range test in which the significance level was defined as p<0.05.

Results and Discussion
The plasma, lung and liver MDA concentration of control rats (at all instances) and of all rats prior to the commencement of the experiment were almost identical (Tables 1-2). Following i.t instillation of BaP and BeP, the
concentration of MDA in the plasma was always higher ($p < 0.05$) in the treated groups compared to the controls. Values returned to normal on day 21 onwards in the BeP group. Similar trends were also seen in the lung and liver. Our results showed that after i.t. instillation, the local and systemic increase of MDA levels (11) indicated that PAHs are absorbed rapidly from the alveolar type I epithelium (12), where it is metabolized locally by phase I enzymes into various polar and water soluble metabolites (12). This study also showed that the response of body to different PAHs varied. This was manifested by the prolonged high MDA levels in the BaP group, where it is diminished on day 21 in the BeP group, giving an explanation why some PAHs could initiate cancer and others not. Important to carcinogenesis, the unregulated or prolonged production of cellular oxidants has been linked to mutation (induced by oxidant-induced DNA damage), as well as modification of gene expression (13).

In conclusion, this study revealed that PAHs differ in their ability to induce MDA, which may lead to different responses to mutagenesis and carcinogenesis.

### Table 1. The plasma MDA concentrations (nmol/ml) of rats during the experimental period (Mean ± SE)

<table>
<thead>
<tr>
<th>Groups</th>
<th>ZERO</th>
<th>12h</th>
<th>32h</th>
<th>3d</th>
<th>7d</th>
<th>21d</th>
<th>60d</th>
<th>180d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cont</td>
<td>1.66±0.07 aA</td>
<td>1.71±0.06 aA</td>
<td>1.64±0.03 aA</td>
<td>1.70±0.08 aA</td>
<td>1.74±0.06 aA</td>
<td>1.71±0.05 aA</td>
<td>1.69±0.05 aA</td>
<td>1.68±0.08 aA</td>
</tr>
<tr>
<td>Bap</td>
<td>1.68±0.05 aA</td>
<td>2.65±0.08 bA</td>
<td>2.73±0.05 bA</td>
<td>2.74±0.05 bA</td>
<td>2.77±0.04 bA</td>
<td>2.79±0.10 bA</td>
<td>2.83±0.04 bA</td>
<td>3.16±0.08 bA</td>
</tr>
<tr>
<td>BeP</td>
<td>1.76±0.06 aA</td>
<td>1.97±0.15 cA</td>
<td>2.32±0.19 bA</td>
<td>2.59±0.18 bA</td>
<td>2.54±0.21 bA</td>
<td>2.01±0.20 cA</td>
<td>1.77±0.07 aA</td>
<td>1.71±0.04 aA</td>
</tr>
</tbody>
</table>

Values bearing similar superscripts in the same row do not differ at $p < 0.05$.

Values bearing similar superscripts in the same column do not differ at $p < 0.05$.

### Table 2. The pulmonary and hepatic MDA concentrations (nmol/mg.protein) of rats at post mortem (Mean ± SE)

<table>
<thead>
<tr>
<th>Groups</th>
<th>12h</th>
<th>3d</th>
<th>7d</th>
<th>21d</th>
<th>60d</th>
<th>180d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>Cont</td>
<td>5.95±0.45 aA</td>
<td>5.89±0.50 aA</td>
<td>6.24±0.49 aA</td>
<td>6.14±0.58 aA</td>
<td>5.94±0.50 aA</td>
</tr>
<tr>
<td></td>
<td>Bap</td>
<td>9.86±0.95 bB</td>
<td>10.02±0.64 bB</td>
<td>10.22±0.58 bB</td>
<td>10.12±0.97 bB</td>
<td>10.11±0.90 bB</td>
</tr>
<tr>
<td></td>
<td>BeP</td>
<td>7.83±0.87 bB</td>
<td>7.53±0.55 bB</td>
<td>7.45±0.71 bB</td>
<td>7.11±0.88 bB</td>
<td>6.34±0.64 aA</td>
</tr>
<tr>
<td>Liver</td>
<td>Cont</td>
<td>15.35±1.88 aA</td>
<td>14.92±2.51 aA</td>
<td>15.25±2.16 aA</td>
<td>18.29±2.61 aA</td>
<td>17.93±1.94 aA</td>
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<tr>
<td></td>
<td>Bap</td>
<td>26.52±3.79 bB</td>
<td>29.05±3.62 bB</td>
<td>28.10±2.83 bB</td>
<td>30.15±3.57 bB</td>
<td>30.53±3.89 bB</td>
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<tr>
<td></td>
<td>BeP</td>
<td>21.06±3.28 bB</td>
<td>21.83±2.94 bB</td>
<td>20.48±3.65 bB</td>
<td>21.18±2.95 bB</td>
<td>19.14±2.29 aA</td>
</tr>
</tbody>
</table>

Values bearing similar superscripts in the same row do not differ at $p < 0.05$.

Values bearing similar superscripts in the same column do not differ at $p < 0.05$.

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### References

Salivary Adenocarcinoma with Splenic Metastasis in a Dog

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Keywords: adenocarcinoma, dog, metastasis, salivary gland, spleen

Introduction
Salivary gland tumors were rarely reported in dogs and cats with over all incidence of 0.07% (1). The salivary tumors generally occur in submandibular and parotid glands (2). Acinic cell carcinoma and adenocarcinoma have been known to occur most frequently in malignant salivary gland tumors. To date, 23 cases of salivary gland adenocarcinoma were reported, 11 in the parotid gland, 5 mandibular, 3 sublingual, 2 pharynx and 1 involving both parotid and mandibular glands in dogs (3). However, to author’s knowledge, there was no report of salivary gland adenocarcinoma with splenic metastasis so far. Here, we describe a case of salivary adenocarcinoma with splenic metastasis in a dog.

Material and Methods
An 8 year-old, female, Maltese dog had a subcutaneous firm mass on left cervical regions. In physical examination, the dog showed severe abdominal pain. In abdomen ultrasonography, hypoechoic lesions were observed in the spleen. Serum biochemistry revealed abnormally increased AST and ALT level. Fine needle aspiration of the cervical mass exhibited undifferentiated glandular epithelial cells showing anisokaryosis and a high nuclear-to-cytoplasmic ratio. A week later, the symptoms of the dog got more severe, which showed spasticity, more enlarged cervical mass, more severe pain and gasp. Finally, the dog was euthanized with owner’s agreement. The organ tissue was sectioned in thickness of 4um and stained with hematoxylin and eosin (HE) and immunostained using antibodies of CK8/18 and CK19.

Results and Discussion
In necropsy, the dog showed enlarged and firm left submandibular salivary gland. There was also a mild enlargement of the adjacent submandibular lymph nodes whereas right submandibular salivary glands and lymph node showed no abnormal lesions. We also observed severely enlarged mesenteric lymph nodes and whitish foci in the spleen and liver. Other organs showed no abnormal lesions. On cut section of the affected organs, each organ showed homogeneous whitish component suspected as a metastasis of tumor cells. Microscopically, the left submandibular gland was replaced by the tumor cells completely. The tumor cells showed prominent, round, vesicular nuclei and scant cytoplasm. The tumor cells also had polygonal shape and arranged in narrow trabeculae, cords and solid sheets separated by fibrous stroma. Small area exhibited an occasional formation of acinar structures which revealed the tumor cells is glandular components. In submandibular lymph node, mesenteric lymph nodes, liver and spleen, the metastasis of the tumor cells were observed. In immunohistochemistry using cytokeratins (CK8/18, CK19) for epithelial marker, the tumor cells showed strong positive reaction, which demonstrated the origin of tumor cells is epithelial component. On physical examination, salivary gland adenocarcinomas are generally firm, painless and attached to deeper structures in the neck, the base of the ear (parotid gland), the upper neck (mandibular gland), the floor of the mouth (sublingual gland) and in the area of lip and maxilla (zygomatic gland) (4). These neoplasms show generally locally invasion, therefore they can extend to the adjacent tissues and distant organs (4). Although salivary gland adenocarcinomas occupy the major part of the salivary gland tumors in animals, the histological features of metastasis of tumor cells to the spleen confirmed with immunohistochemistry was not reported to date.

Fig. 1 (a) Cut surface of the affected salivary gland. Basophilic neoplastic tumor cells replacing the right submandibular gland (b) (x100, HE), left submandibular lymph node (f) (x200, HE) and spleen (j) (x200, HE). (c) Enlarged left submandibular lymph node (arrow). (i) Whitish metastatic lesion on cut section of the spleen. CK8/18 and CK19 positive tumor cells in salivary gland (c, x400, d, x200), lymph node (g, h, x400) and spleen (k, l, x400).

References
Ossifying Fibroma of External Auditory Canal in a Dog


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Keywords: aural, dog, external auditory canal, ossifying fibroma

Introduction

Ossifying fibroma is an uncommon benign-osseous neoplasm in humans and animals (1, 2). Histological feature of ossifying fibroma shows intermediate stage between osteoma and fibrous dysplasia (3). In veterinary literature, ossifying fibromas have been reported in dogs, cats, horses, a greater kudu, a goat, a sheep, a llama (4), a rabbit (5) and a bird (6). The mandible and maxilla were known as the most common places in which ossifying fibromas have occurred in animals (4). To date, in dogs, ossifying fibroma was reported only in the maxilla, mandible, calvarium and os penis (3). The present case describes a first report of ossifying fibroma occurred in external auditory canal in a dog. Ear canal tumor is also relatively uncommon in dogs, which was mainly associated with ceruminous gland tumors (7).

Material and Methods

A 3 year-old, male, Pomeranian dog was presented a local veterinary hospital because of a hemorrhagic externa otitis of right ear. In physical examination, the dog appeared to have no abnormal symptom. Serum biochemistry and complete blood count test results showed normal range. Upon otoscopy, the right external auditory canal was revealed to be almost obstructive by a proliferative and well-circumscribed mass adjacent to the ear drum. In cytological examination, the spindle-shaped stromal cells with oval nuclei and scant cytoplasm were observed. Surgically, the ear canal mass was excised and the mass was referred. The resected mass was sectioned in thickness of 4um and stained with hematoxylin and eosin (HE) and Massonís trichrome stain.

Results and Discussion

Grossly, the ear canal mass was well-demarcated and firm to cut. On cut section, the mass exhibited central portion of homogeneous whitish osseous components surrounded by brown-pinkish soft tissue. Histopathologically, the resected external auditory canal mass was composed of fibroblastic spindle cells which showed differentiation to metaplastic osteoblasts-like cells. The metaplastic osteoblasts formed and surrounded osteoid bony spicules. The bony spicules were separated by abundant collagens and neoplastic fibroblastic cells. The fibrous stroma of the mass was moderately vascularized and exhibited mild infiltration of neutrophils. Neoplastic spindle cells were characterized by elongated ovoid nuclei, scant cytoplasm and indistinct cell borders. Mitotic figures were not observed. Some parts of the mass adjacent to bony spicules exhibited the presence of osteoclasts. In Massonís trichrome stain, the bony spicules were differentiated clearly.

Osteoma, ossifying fibroma and fibrous dysplasia belong to a miscellaneous group of benign lesions found primarily in intramembranous bone (1). Ossifying fibromas generally show a greater density of fibroblastic spindle cells and fibers in tissue spaces between bony trabeculae than that of the marrow space of osteomas, which distinguishes ossifying fibroma from osteoma (1). To the best of author’s knowledge, ossifying fibroma of external auditory canal was not reported in not only animals including dogs but also human beings.

References

Subcutaneous Leimyosarcoma in a Smad3 Hetero Mouse

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Key words: alpha-smooth muscle actin, leimyosarcoma, mouse, smad3

Introduction

Superficial leiomyosarcoma, which can be divided into two groups; subcutaneous and cutaneous leimyosarcoma, is a rare malignant tumor in both humans and animals and thought to originate from the smooth muscle cells in blood vessels and the arrectores pilorum (1-4). This Study is a case of subcutaneous leiomyosarcoma in a Smad3+/- mouse, which presented a mass in the ear. Smad3 is a key modulator of the TGF-β signaling pathway and is related broadly with gene expression. For that reason, Smad3 transgenic mice have been widely used and have played a critical role in the research field (5, 6).

Material and Methods

The Smad3 mutant mice were obtained from the National Cancer Institute, MD, USA, and kept in a room at 22±2% with 50±10% of relative humidity on a 12 h light-dark cycle and fed standard laboratory feed and water ad libitum. After the 19-month-old ear tumor presenting male Smad3+/- mouse died, PCR was performed to confirm the genotype of Smad3+-. A tumor sample was fixed in 10% neutral buffered formalin, routinely processed, embedded in paraffin, sectioned to 4um thickness and stained with H&E. After the tumor type was determined by H&E, IHC was performed on the paraffin sections using an avidin-biotin-peroxidase complex method with 3, 3-diaminobenzidine. Anti-vimentin, anti-cytokeratin, anti-smooth muscle actin, anti-desmin, anti-myogenin and anti-S100 were used for IHC.

Results and Discussion

The auricular skin tumor was firm and the gross lesion of the cut surface showed a whitish component with reddish hemorrhaging. The histopathologic examination showed nonencapsulated partially invasive spindle to ovoid cells. Among the mixture of well-differentiated and less differentiated cells, mitoses were present (1-2 mitoses/
404x field), evidence of invasion to the adjacent skeletal muscle, loss of skin adnexa, and necrosis were shown. The tumor was apt to form a cyst and epithelial cells showed inward hyperplasia. The IHC analysis of the mass shows a strong diffuse positive reaction for desmine and a-SMA, and a weak but partially strong positive vimentin in the cytoplasm of the neoplastic cells. However, reaction for CK, S100, and myogenin were all negative. Consequently, the present case was diagnosed as a subcutaneous leiomyosarcoma via the results taken from the gross lesion, histopathologic examination and immunohistochemistry.

The genotyping result from the mouse with subcutaneous leiomyosarcoma revealed 431bp, 284bp PCR products, which confirmed it was a Smad3+/- mouse. Some of important roles of Smad3 are the G1 arrest of cell cycle, apoptosis, and recruiting inflammatory cells during the wound healing and suppression of Smad3 pathway can induce carcinogenesis (7, 8). The present case also shows some features which is related with Smad3 mutant such as epidermal hyperplasia, which usually arise form cutaneous tumor, and suppression of inflammation in an injured lesion (9).

Leiomyosarcoma of the skin and subcutis in animals reported extremely rarely and this is the first report of subcutaneous leiomyosarcoma in a Smad3 transgenic mouse to the author’s knowledge.

Fig. 8 Necrosis lesions in leiomyosarcoma (H&E, x400)
Fig. 9 Immunohistochemistry for vimentin (x400)
Fig. 10 Immunohistochemistry for desmin (x400)
Fig. 11 Immunohistochemistry for α-SMA (x400)

References
2. Rouhani et al., 2008. Cancer. 113: 616-627
8. Li et al., 2006. Mol. Carcinog. 45: 389-396
Porcine Multicentric B-cell Lymphosarcoma in Korea

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Keywords: B cell, Korea, lymphosarcoma, porcine

Introduction

Lymphosarcoma is considered as a rare porcine disease but, common of all tumors (1). It primarily affects young animal before maturity but also in mature animal (2). It is known that lymphosarcomas are classified into three forms: multicentric, thymic and cutaneous form. In pig, most cases are the multicentric form (3). It has been reported from many countries, but in Korea, lymphosarcoma has been rarely reported (4). So we report a case of porcine multicentric B cell lymphosarcoma in Korea.

Material and Methods

A six-month boar died and autopsy was done with gross examinations. Tumor mass, the liver, lung, kidney, spleen and mesentery lymph node were taken for histological examination. Samples were fixed in 10% neutral buffered formalin, routinely processed and embedded in paraffin wax. Sections were cut out at 4 μm. The sections were stain with hematoxylin and eosin. Immunohistochemistry using CD3 and CD79a was done for differential diagnosis of B cell lymphoma and T cell lymphoma.

Results and Discussion

Grossly, large tumors were observed on the surface and the mesenteric lymph nodes were enlarged. The cut surface revealed homogenous whitish color. The liver showed many whitish nodules.

Fig. 1 A) Large tumor masses of intestine. B) Liver showed many whitish nodules.

Fig. 2 A) Tumor mass showed many lymphoblastic cells, mitotic figure and apoptotic body (x400). B) Hepatocytes were degenerated by infiltrated neoplastic cells (x400).

Fig. 3 The tumor cells showed a positive reaction with CD79a

showed many nodular masses. Masses were whitish and discovered in all liver parenchyma surfaces. The liver was almost destructed by mass showing sever invasions. The lung exhibited mild swelling and had multifocal whitish nodules in the right cranial lobe. On cut surface of the lung, the lung parenchyma was filled with yellowish exudates. The kidney had normal size and pale color. When cut the kidney, cortex was yellowish. There was no significant findings in the spleen.

Microscopically, the mesenteric lymph node parenchyma was infiltrated by many neoplastic cells and filled with homogenous lymphoblastic cells. There were many mitotic figures and apoptotic bodies. In the liver,
many neoplastic cells also infiltrated into hepatic sinusoid and hepatocytes, and hepatic lobules were almost degenerated by the neoplastic lymphoblastic cells. The lung showed mild edema and exhibited slightly interstitial pneumonia. However, there was no tumor cell metastasis. In the kidney, there were mild atrophic glomerulus but tumor metastasis was not observed.

Immunohistochemistry was performed with CD3 for T cell marker and CD79a for B-cell marker. In the present case, the tumor cells showed a positive reaction with CD79a and negative reaction with CD3.

Taken all together, the diagnosis of is porcine multicentric B cell lymphosarcoma. It is known that multicentric form lymphosarcoma involve many organs from many cases. However unlike most cases, this case showed only hepatic metastasis.

Porcine lymphosarcoma was rarely reported in Korea. In 2007, it was reported that lymphosarcoma occurred in Jeju, Korea. In the case, animal was 7-year-old boar and diagnosed as multicentric T cell lymphosarcoma. According to the author’s knowledge, it is the first porcine multicentric T cell lymphosarcoma in Korea (4). However, unlike that case, our animal was six-month boar and multicentric B cell lymphosarcoma.

Although, porcine lymphosarcoma is rare in Korea, it has been reported from many countries. Therefore, it is necessary for Korean clinical pig veterinarian to have more attention to the lymphosarcoma in pig.

References
Bilateral Extranodal Lymphoma of the Third Eyelid Conjunctiva in a Dog


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Keywords: dog, lymphoma, third eyelid

Introduction
The third eyelid is modified conjunctival fold in the medial canthus of some animals. Although the third eyelid is small ocular adnexa, various diseases including the tumors have been reported. Tumors of the third eyelid are rare in dogs, however, primary neoplasms, metastatic tumors, tumors extending from adjacent structures, and systemic neoplasms can affect the third eyelid. Squamous cell carcinoma, melanoma, papilloma, fibroma, hemangioma, lipomas, mast cell tumors, adenoma and adenocarcinoma have been reported in the third eyelid as primary neoplasms (1). Secondary involvement is most commonly a consequence of multicentric lymphoma. However, extranodal lymphoma of ocular conjunctiva is rare, moreover the third eyelid involvement has not been reported yet to the author's knowledge. In the present paper describes the clinical and histopathologic findings of bilateral extranodal lymphoma of the third eyelid in a dog.

Material and Methods
A 4-year-old, neutered female Cocker spaniel was presented with protrusion of third eyelid of left eye. The owner reported that protrusion was observed 1 month prior to examination. The mass was excised under general anesthesia and then referred to histological examination. The mass was fixed in 10% neutral buffered formalin, routinely processed and embedded in paraffin. Sections were cut at 4 μm and stained with hematoxylin and eosin (H&E). Immunohistochemistry for CD3 (T lymphocyte marker) and CD79a (B lymphocyte marker) were executed to define immunophenotype of the cells.

Results and Discussion
On ocular examination, the third eyelid was protruding, thickened and hyperemic (Fig. 1A). When the third eyelids from both eyes were reversed, an increase of volume of its internal surface could be verified. The third eyelid of left eye displayed a lobulated mass (0.5x0.3 cm) (Fig. 1B). The right third eyelid had a smaller one (0.2x0.2 cm) not easy to detect by owner. There were no significant findings except for both third eyelid lesions and no regional or generalized lymphadenopathy. In histological examination, the mass was consists of multiple lymphoid follicle proliferation, and lymphoid cells infiltrated into subconjunctival connective tissue. The lymphoid follicles were consisted of small and large lymphocytes with a lighter center and narrow darker mantle zone (Fig. 2A). There were observed a few apoptotic bodies with karyopyknotic and karyorrhexic nuclei in germinal center. Mitotic figures were rare. In immunohistochemistry, almost cells in lymphoid follicles and infiltrated cells into the connective tissue in the mass were expressed for CD79a not CD3 (Fig. 2B). The tumor and the third eyelid of left eye were removed for biopsy and right one was remained. Topical therapy was prescribed with neomycin and dexamethasone ointment (Forus, Samil Pharm, Korea). At 1 year follow-up, the tumor of right third eyelid was still remained as similar size. However, the dog still showed no significant findings except those tumor with no evidence of systemic involvement.

Diffuse lymphoid follicles are normally located under the bulbar conjunctival surface of the third eyelid, and they have immunological activity. Lymphoid hyperplasia (follicular conjunctivitis) is very common disease of the third eyelid. It is due to chronic irritation and immunological stimulating proliferation of the normally present follicles on the bulbar aspect of the third eyelid. Therefore, lymphoid hyperplasia should be differential diagnosis to lymphoma in third eyelid. In the third eyelid of normal healthy dogs, B lymphocytes located in germinal centers of the lymphoid follicles and T lymphocytes are surrounding the B cell dominant germinal centers. Moreover, our present case showed tumorous features including appearance of a few apoptotic cells and mitotic figures. Therefore, a diagnosis of lymphoma on third eyelid (follicular mixed cell type, low grade) was established based on the histological and immunophenotypical features. To the author's knowledge, this is the first report of extranodal lymphoma of the third eyelid in a dog.

Fig. 1 Left eye of a dog (A) and formalin-fixed tumor (B)

Fig. 2 H&E stain, x200 (A) immunohistochemistry of CD79a, x200 (B).

References
A First Case Report of Histoplasmosis in a Cat in Japan

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Keywords: cat, histoplasmosis, intestine

Introduction

Histoplasmosis is caused by *Histoplasma capsulatum* (*H. capsulatum*), which is a dimorphic soil-borne fungus. In disseminated histoplasmosis, in which necrotizing and granulomatous inflammation develop observed in various systemic organs. There have been no reports of feline histoplasmosis in Japan. In this report, we describe cytological, histopathological, and immunohistochemical findings of intestinal histoplasmosis in a cat. This is the first report of feline histoplasmosis in Japan.

Material and Methods

A presumed 10-year-old, spayed female, domestic Japanese cat was referred to a veterinary hospital with vomiting. Physical examination revealed a palpable mass in the upper abdominal cavity. In cytologic examination of the fine needle aspiration from the mass, many yeast-like organisms were detected in macrophages (Fig 1). These organisms were observed as basophilic dots with a clear halo and measured 2 to 3 μm in diameter. The animal died on the following day. At necropsy, the upper part of the colon was markedly dilated with a thickened wall (Fig 2). The pancreatic lymph node was markedly enlarged and colored dark red. In the lungs, no significant changes were observed. The tracheobronchial lymph node was enlarged and firm. Tissue samples were collected from systemic organ for histopathological examination. For the immunohistochemistry, rabbit anti-histoplasmal yeast antibody (Meridian Diagnostics, Inc., Cincinnati, OH, U.S.A.; 1:1,000) were used as primary antibodies.

Results and Discussion

Microscopically, severe necrotizing and granulomatous inflammation was observed in the wall of the dilated colon (Fig 3), in the pancreatic lymph node, and in the tracheobronchial lymph node. In the dilated wall of the colon, there were many macrophages containing yeast-like bodies as irregularly shaped eosinophilic round structures throughout the necrotic lesions. Immunohistochemically, these organisms were positive for rabbit anti-histoplasmal yeast antibody (Fig 4). The lesions accompanying the yeast-like organisms were not seen in any other organs. The cytological, histopathological and immunohistochemical findings suggest that the cause of the intestinal lesion might be *H. capsulatum*.

Acknowledgements

We are indebted to Prof. Hiroyuki Taniyama (School of Veterinary Medicine, Rakuno Gakuen University) for his kind gift of the anti-histoplasmal yeast antibody.
Effect of Serum Cortisol and Progesterone on the Infiltration of Leukocyte Subpopulations in the Gilt Endometrium

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Keywords: cortisol, endometrium, gilt, progesterone, reproduction

Introduction

The reproductive function of the female pigs is difficult to examine under field condition. Post-mortem examination of the reproductive organs is a useful tool to obtain a potential source of information on infertility problems (1-4). Factors causing reproductive failure alter physiological status of the sow’s endometrium in different pathways (5). However, the infiltration of immune cells in cyclic gilts and sows is also influenced by the estrous cycle and hormones (6-7). Progesterone (P4) increases tissue proliferation, gland development and protein secretion in the porcine endometrium (6). In addition, P4 increases the susceptibility of the endometrium to bacterial infection and may subsequently cause endometritis (8). The infiltration and distribution of leukocytes in the porcine endometrium during the estrous cycle have been comprehensively evaluated (6-7). Lymphocytes are the predominant population in the endometrium of the cyclic gilts, while the striking observation of numerous neutrophils was found in the endometrium of pre-pubertal gilts (6-7). The influence of stress on the reproductive function is well established (9). In pig, cortisol is an important hormone linked between stress and reproductive functions (9). However, information on the influence of cortisol on the distribution of immune cells in the uterine tissue of gilts and sows is limited. The present study was performed to evaluate the influence of cortisol and P4 on the distribution of leukocyte subpopulations in the giltis endometrium.

Material and Methods

Tissue collection: Genital organs from 39 Landrace x Yorkshire crossbred gilts culled due to vaginal discharge from two commercial swine herds in Thailand were collected. The organs including ovary, oviduct, uterus, cervix, vagina, vestibule, vulva and urinary bladder were collected, placed on ice, and transported to the laboratory within 24 h of culling. These organs were examined to assess the stages of the estrous cycle and gross-pathology (4). Ovarian appearance and component structures, i.e. corpora lutea (CL), corpora albicantia (CA) and follicles, on the ovaries were carefully examined. The uterine horns were opened longitudinally and the endometrium was investigated. Tissue samples were randomly collected from the uterus of the gilts including two parts of each uterine horn. The samples were fixed in 10% neutral buffered formalin for at least 24 h, embedded in paraffin, and processed by use of an automatic tissue processor. Each sample was embedded in paraffin block using embedding instrument. The paraffin embedding was cut with 5 μm thickness by microtome. The slides were left overnight at 37°C. The tissues were deparaffinized using xylene, passed different concentration of alcohol and were stained using haematoxylin for 5 min and eosin for 30 sec (H&E).

Histological examination: The sections were divided into three layers for histological examination, i.e. epithelial, subepithelial connective tissue and glandular layers. Immune cells, i.e. lymphocytes, neutrophils, eosinophils, macrophages and plasma cells in each layer were quantified under light microscope (400x). For each section and each layer, 20 microscopic fields were arbitrarily selected for investigation. Ocular micrometer with 25 squares corresponded to 15,625 μm² (400x) of real tissue area and 125 μm of real tissue length was used for counting the number of immune cells in each area by movement of the ocular micrometer across the entire area in a non-overlapping manner (6). The number of immune cell counts was expressed as the total number of cells per uterine section (20 microscopic fields).

The degree of endometritis was categorized into mild, moderate, and severe condition on the criterion of neutrophil number infiltrated into the giltis endometrium. In the follicular phase, endometrium having neutrophils <80, 120, and >120 cells/ 20 microscopic fields, <9, 15, and >15 cells/ 20 microscopic fields in the luteal phase, and <2, 3, and >3 cells/ 20 microscopic fields in the inactive phase, were considered mild, moderate, and severe endometritis respectively (12).

Hormonal analyses: Blood samples were collected from the jugular vein prior to slaughter. They were centrifuged at 1,160x g for 10 min. The sera were collected and stored at -20°C until assay. Serum P₄ was analyzed by enzyme immunoassay (EIA) using a P₄ monoclonal antibody (CL425) (10). Briefly, plates (NUNC, Maxisorb), except for nonspecific binding wells, were coated with 50 μl of antibody (1:7,500) and incubated overnight (12 h) at 4°C. Plates were washed 5 times and 50 μl of P₄ standard (0.78-200 pg/well), control and serum samples were added, followed immediately by 50 μl of P₄-horseradish peroxidase (1:65,000). Plates were incubated at room temperature for 2 h followed by addition of 100 μl of ABTS substrate (40 μl 0.5 M H₂O₂, 125 μl 40 mM ABTS in 12.5 ml...
0.96% citric acid solution). Plates were read at 405 nm (ELISA reader, TECAN SUNRISE, Austria). Optical density (OD) for 0 wells was >0.7 to <1 OD. Assay sensitivity at 90% binding was 0.016 ng/well. The intra assay CV for low and high controls was 6.15% and 9.05%, respectively. The serum cortisol level was determined by enzyme immunoassay (EIA) (Active® Cortisol EIA kit, Diagnostic Systems Laboratories, Inc., Texas, USA). The assay was performed according to the manufacturer’s instructions. The principal of the procedure follows the basic principal of EIA, which there is competition between an unlabeled antigen and an enzyme labeled antigen for a fixed number of antibody binding sites. A known amount of cortisol was added to the assay in order to calculate the intra-assay coefficients of variation, which were 0.84% and 0.33% for low and high cortisol concentration, respectively.

**Statistical analyses:** Data were analyzed using SAS (SAS v. 9.0, Cary, NC, USA). Numbers of cells were presented as the mean number of cells per 20 ocular fields (312,500 μm²) in four tissue sections. The data were analyzed using general linear model procedure (PROC GLM). Normal distribution of the data was tested using the UNIVARIATE procedure. A natural logarithmic transformation was applied to the number of immune cells. Least-squares means were obtained and were compared using least significant different test. Spearman’s correlation was used to analyze the association between cortisol, P₄, and the number of leukocyte subpopulation. p ≤0.05 was regarded to have statistical significance.

**Results and Discussion**

From the appraisal of genital organs, the number of gilts in follicular, luteal, and inactive phases was 10, 25, and 4 respectively. On average, cortisol concentration in the slaughtered gilts was 430.6±68.3 nmol/l (range 46.6-1,656.0). P₄ varied according to the ovarian appearance. On average, P₄ concentration was 88.3±7.7 nmol/l during luteal phase, 20.6±6.2 nmol/l during follicular phase, and 18.5±14.7 nmol/l during inactive phase. The most obvious leukocyte subpopulation in normal gilts, mild, and moderate endometritis was lymphocytes (in epithelium, subepithelial connective tissue, and glandular layers: 16.4, 88.8, and 50.9 cells in normal gilts, 34.3, 58.1, and 38.63 cells in mild endometritis gilts), meanwhile, neutrophils were regarded as the most apparent subpopulation in gilts with severe endometritis (46.6, 126.5, and 23.0 cells in epithelial, subepithelial connective tissue, and glandular layers respectively). Vividly, the subepithelial connective tissue layer was the most prominent area in which the leukocytes infiltrated. The number of leukocyte subpopulations of the endometrium and the concentrations of cortisol and P₄ classified by degree of endometritis in subepithelial connective tissue layers are demonstrated in Table 1. The gilts having severe endometritis tended to have a higher cortisol level than the gilts having normal endometrium (p=0.07).

In the epithelial layer, negative correlation between P₄ and neutrophils was observed (r=-0.44, p=0.006). In the subepithelial connective tissue layer, P₄ was negatively correlated with neutrophils (r=-0.45, p=0.004) and positively correlated with eosinophils (r=0.46, p=0.003). Serum cortisol was negatively correlated with lymphocytes in the subepithelial connective tissue layer (r=-0.2, p=0.08) and in the glandular layer (r=-0.2, p=0.09).

It is well-established that cortisol responses to stress and causes immunosuppression (9). In the present study, high cortisol concentration tended to decrease number of lymphocyte in subepithelial connective tissue and glandular layers of the gilts. Brandt et al. (11) demonstrated that the sows injected by ACTH had far higher level of serum cortisol (336±55 nmol/l) than the NaCl-injected (85±15 nmol/l) sows. The cortisol level in the present study (430.6±68.3 nmol/l) corresponded with the ACTH-induced sows of Brandt et al. (11). This implies that the endometritis gilts are stressful as indicated by the high level of serum cortisol. The correlation between serum cortisol and lymphocytes was apparently negative which seemed to determine the immunosuppressive condition.

**Table 1** Levels of serum cortisol (nmol/l), P₄ (nmol/l) and number of leukocyte subpopulations in the subepithelial connective tissue layers of the gilts endometrium by degree of endometritis (mean±SEM)

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**Acknowledgement**

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**References**

The Expression of Caspase 3 in the Chicken Bursa Cell in Tasik’98 Infectious Bursal Disease Virus Infection

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Keywords: apoptosis, chicken, infectious bursal disease

Introduction

Infectious bursal disease (IBDV) is a highly contagious viral infection of chickens that is seen worldwide. The bursa of Fabricius is the primary target organ of IBDV (4). The virus replicates in immature bursa-derived lymphocytes (B-lymphocytes). The chickens which survive the disease are permanently immunosuppressed. Therefore they are more susceptible to other disease causing agents and don’t respond adequately to vaccinations which are an essential part of poultry management systems (6, 7). Virulence of field strains of the virus varies considerably. Very virulent (vv) strains of the virus that cause high mortality and morbidity were detected first in Europe. These spread throughout the Old World in the last decade and in 1999 were in South America. Infection by this virus due to depletion of lymphocyte in the bursa of Fabricius because of necrosis and apoptosis (5, 8). Tasik’98 is one of vv IBDV field strain from Indonesia, which can cause an acute clinical disease characterized by devastating mortality and the severe depletion of bursa of Fabricius.

The main purpose of this study was to proof the increasing of bursa cells apoptosis by detection of Caspase 3 expression in bursa of Fabricius. The Caspase 3 is a main effector caspase in apoptosis process. Direct or indirect activation of caspase 3 are responsible for target proteine cleavage due to a specific DNA and also in specific morphologic changes of apoptotic cells (1). When caspase 3 has activated, the cell committed to death and the apoptosis may called due to the point of no return (3, 4). The expression of activated caspase 3 in the cell occured at the early apoptosis process before appears clasical morphologic changes of cell death, so detection of the increasing of caspase 3 expression in the cell by immunohistocemestry can be used as the base for detection of apoptotic cell.

Material and Methods

Animal trial in this study were specific pathogenic free day old chick. Virus that used for infection was Tasik ’98 isolate IBDV.

The study consists of two parts. Forty-four chickens on the first step of experiment are devided into 2 groups. Chickens in Group I were infected with 1000 TCID 50 of Tasik’98 isolate IBDV through intraocular, peroral, and intracloacal routes. Chickens in group II were treated with NaCl through intraocular, peroral and intracloacal routes as placebo. Randomly, each two chickens from 2 groups, were sacrificed on 12, 14, 16, 18, 20, 22, 24, 48, 72, 96, 120 day post infection. The bursa was collected and fixed in formalin buffer for histologigy and immunohistochemistry. Primary antibody was monoclonal rabbit anti caspase 3 antibody (LabVision Corp.). Caspase 3 expression was evaluated by counting the number of positive cells in percent. The time when caspase 3 expression increases for 25% will be deter-mination of a time for sacrificing chicken in the second step of experiment.

Sixteen chickens on the second step of experiment are divided into 2 groups. Chickens in Group I were infected with 1000 TCID 50 of Tasik’98 isolate IBDV through intraocular, peroral and intracloacal routes. Chickens in group II were treated with NaCl by intraocular, peroral and intracloacal routes as placebo. On the time that was determined in first step of experiment, all of the chickens were sacrificed. The bursa were collected for histology and were evaluated on Caspase 3 expression used the method like the one that was done in
the first step of experiment above. The data were analysed by student t test.

Results and Discussion

The result of first step experiment showed that the 25% increasing of caspase 3 expression were found on 48 hours post infection. It was used for determination for sacrificing chicken in the second step of experiment.

The result of the second experiment showed that the expression of caspase 3 in bursa were significant difference ($p<0.05$) between infected chicken and non-infected chicken. Expression of caspase 3 increased from $1.25\pm1.05\%$ in the non infected chickens became $29.94\pm2.01\%$ in the infected chickens like showed in Fig. 1.

![Fig 1](image)

Caspase 3 was strongly expressed in infected chicken bursa (a, c) and there is almost negative in uninfected chicken bursa (b, d).

There are three apoptosis mechanisms that involve caspase activation. The first, granzyme/perforin mediated pathway. The second is dead receptor mediated pathway. Fas is one of the dead receptor, it was expressed in the cell membrane surface when the cell has been infected by a virus. The third, cytocrom c released from mitochondria bind Apaf-1 can induce the activation of caspase 9 (caspase initiator). Caspase 9 will activate the effector caspase due to apoptosis (1, 4).

The mechanism of bursal apoptosis in infectious bursal disease virus infected chicken still has not been fully understood. It was thought that the mechanism trough the Fas-Fas ligand pathway that could be explained with increasing of Fas and caspase 3 expressions, or perforin-granzyme pathway that could be explained with increasing of granzyme and caspase 3 expressions.

The result of this study showed that there was the increasing of caspase 3 expression in IBDV infected chicken bursa. It means that there was increasing of apoptosis in chicken bursa of Fabricius in Tasik’98 isolate IBDV infection. The increasing of caspase 3 expression could be described that bursa cell contained virus express Fas. Fas will bind Fas ligand of cytotoxic T lymphocyte. This binding will activate Fas associated dead domain and then run to cascade caspase activation. The other ways, activated cytotoxic T lymphocyte will releases perphorin and granzyme. Perphorin makes a pore in the virus infected cell membrane and then the granzyme enter the cell through the pore. Granzyme can activate caspase cascade.

References

Pathological Study on the Pulmonary Toxicity Induced by the Intratracheally Instilled Asian Sand Dust in Mice

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Keywords: acute inflammation, asian sand dust, intratracheal instillation, lung

Introduction
With global climate change, frequency and volume of asian sand dust (ASD), which originates from Gobi desert, are increasing in east Asian region since 2000 (1) resulting in one of the air pollution related threats to both human and animal health. Epidemiological studies showed that ambient ASD particles are associated with an increase in pulmonary and cardiovascular morbidity and mortality in Korea (2) and Taiwan (3).

There are, however, few reports on the pathological study of the pulmonary toxicity induced by ASD. In this study, we examined changes in the bronchoalveolar lavage fluids (BALF) and lung tissues of mice intratracheally instilled with ASD.

Material and Methods
0.05 ml of saline containing 50, 200, 800 and 3000 μg of ASDs (CJ-2 originated from Tengger desert, General Science Corp., Japan) were intratracheally instilled to ICR male mice. 0.05 ml of a saline solution was instilled to control mice. Animals were sacrificed at 24 h following exposures. Bronchoalveolar lavage fluid analysis (cell viability, differential cell counts and total protein concentration), histopathological examination and immunohistochemistry (TNF-α) of the lung tissues were assessed.

Results and Discussion
The intratracheal instillation of 800 and 3000 μg ASD significantly decreased the cell viability compared to the control group. Changes in the increased number of neutrophils and total protein concentration in BALF were dose dependent.

The histopathological examination revealed that ASD-induced severe inflammation around the dusts with neutrophils and macrophages and hemorrhage. In addition, TNF-α immunoreactivity was observed mainly in the inflammatory cells of the legion in lung tissues.

These results suggest that ASD induce acute pulmonary inflammatory changes. Mechanisms of health effect by particulate matter include direct effects of particle components and indirect effects by pro-inflammatory mediators released from particulate matter-stimulated macrophages (4). The inflammatory lung injury in this study may be caused by both ASD particle itself and the cytokines released in the lesion.

Table 1 Bronchioalveolar large fluid (BALF) analysis in mice after exposure to asian sand dust

<table>
<thead>
<tr>
<th>Dose of instillation (μg/animal)</th>
<th>Percentage of neutrophils (%)</th>
<th>Concentration of total protein (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.02±0.05</td>
<td>243.0±47.4</td>
</tr>
<tr>
<td>50</td>
<td>0.14±0.23</td>
<td>275.6±108.4</td>
</tr>
<tr>
<td>200</td>
<td>0.36±0.38</td>
<td>420.1±137.1</td>
</tr>
<tr>
<td>800</td>
<td>14.33±6.40*</td>
<td>1178.5±50.8</td>
</tr>
<tr>
<td>3000</td>
<td>44.38±2.69*</td>
<td>2375.0±116.4</td>
</tr>
</tbody>
</table>

*Significant different from the control group (p<0.05)

Fig. 1 Histopathology of the lungs from mice following the intratracheal instillation of 3000 μg ASDs.
(A) Hematoxylin and eosin stain, (B) high magnification of (A), (C) Expression of TNF-μ in the inflammatory region, (D) High magnification of (C).

In conclusion, this study demonstrated that mineralogical components of asian sand dust particles, free from chemical and biological pollutants in the atmosphere, induce acute inflammatory changes in the lung tissue and BALF in vivo.

References
Pathological Study of Neurodegenerative Process of Equine Motor Neuron Disease

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Keywords: blood spinal cord barrier, equine motor neuron disease, leakage, lipopigment, oxidative stress

Introduction

Equine motor neuron disease (EMND) is a sporadic and progressive neurodegenerative disorder characterized by selective degeneration and loss of motor neuron, gliosis and formation of eosinophilic intracytoplasmic inclusion body in the spinal cord and certain cranial nerve nuclei (1).

Although Vitamin E deficiency is considered as a risk factor of EMND (2), the detail pathological process of neuronal loss in EMND is unknown. Because accumulation of endothelial lipopigment is observed in the small vessels of the spinal cord (3), it is suspected that motor neuron degeneration is mediated by blood spinal cord barrier (BSCB) disruption. This hypothesis is also supported by the recent studies of human motor neuron disease, amyotrophic lateral sclerosis (ALS) which closely resembles EMND (4).

Material and Methods

3 EMND cases and 3 control cases with no neurologic disorder were used. Complete postmortem examination was performed on all horses, and selected tissues were fixed in 10% neutral phosphate buffered formalin. The tissues were routinely processed and stained with luxol fast blue (LFB) staining and Prussian blue-DAB post-DAB enhancement staining (5). Basement membrane degeneration, serum protein leakage and production of free radical were examined by immunohistochemical method. Primary antibodies were polyclonal rabbit anti-fibronectin antibody (ABR), anti-horse IgG antibody (ROCKLAND) and monoclonal mouse anti-metallothionein antibody (DAKO).

Results and Discussion

Grossly, the ear canal mass was well-demarcated and firm to cut. On cut section, the mass exhibited central portion of homogeneous whitish osseous components surrounded by brown-pinkish soft tissue. Histopathologically, the resected external auditory canal mass was composed of fibroblastic spindle cells which showed differentiation to metaplastic osteoblasts-like cells. The metaplastic osteoblasts formed and surrounded osteoid bony spicules. The bony spicules were separated by abundant collagens and neoplastic fibroblastic cells. The fibrous stroma of the mass was moderately vascularized and exhibited mild infiltration of neutrophils. Neoplastic spindle cells were characterized by elongated ovoid nuclei, scant cytoplasm and indistinct cell borders. Mitotic figures were not observed. Some parts of the mass adjacent to bony spicules exhibited the presence of osteoclasts. In Masson’s trichrome stain, the bony spicules were differentiated clearly.

Osteoma, ossifying fibroma and fibrous dysplasia belong to a miscellaneous group of benign lesions found primarily in intramembranous bone (1). Ossifying fibromas generally show a greater density of fibroblastic spindle cells and fibers in tissue spaces between bony trabeculae than that of the marrow space of osteomas, which distinguishes ossifying fibroma from osteoma (1). To the best of author’s knowledge, ossifying fibroma of external auditory canal was not reported in not only animals including dogs but also human beings.

Fig. 1 Luxol fast blue staining (A), Prussian blue-DAB post-DAB enhancement staining (B), Immunostaining for IgG (C), Immunostaining for fibronectin (D), Immunostaining for metallothionein antibody (E).

References

Cerebellar Ataxia Induced by Plant Toxicosis in a Goat in Mongolia

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Keywords: ataxia, cerebellum, goat, plant toxin, Purkinje cell

Introduction
With global climate change (abnormal climate, warming temperature, drought) and human activity (over depasturaging, water pollution), vegetation dynamics are rapidly changing in Mongolia resulting in overgrowth of particular poisonous plants in the pasture field. In June 2009, a locomotor disorder developed in some goats that had grazed Oxytropis glabra in the Khovd province of Mongolia (Fig. 1). Affected goats displayed neurological signs including limb paresis, knuckling over in the fetlocks, fine head tremor, incoordination and an equilibrium disturbance characterized by frequent falling. Epidemiology indicates that the ingestion of Oxytropis glabra, by which plant Mongolian pasture has been replaced in the past 10 years by the significant environmental changes, is involved in the etiology of the disease. Here, we describe pathological findings of a goat with cerebellar ataxia in Mongolia.

Material and Methods
A goat with cerebellar ataxia was euthanased and necropsied. The brain and other organs (liver, spleen, kidney, heart, lung) were corrected, and tissues were fixed in formalin and embedded in paraffin. Sections were cut and stained with hematoxylin and eosin.

Results and Discussion
No gross pathological changes were seen in the goat. The microscopic examination of the cerebellum demonstrated decreased number of Purkinje cells (Fig. 2), increased number of glial cells (astrocytes and microglia) in the medulla (Fig. 3). These were no significant change in the other organs.

Purkinje cell loss and the gliosis present in the cerebellum were prominent. These pathological changes are in agreement with clinical neurological symptoms. Cerebellar ataxia due to plant poisoning was reported in sheep in Australian Merinos, goats grazing Solanum cinereum in Australia and S. viarum in the United States, and cattle grazing S. kwenense in South Africa, S. dimidiatum in the United States, S. fastigiatum in Brazil, and S. bonariensis in Uruguay (1). This is the first report of cerebellar ataxia in animals ingested Oxytropis glabra. Identification of the toxin and study on the mechanisms of Purkinje cell loss are required. The disease observed in the domestic animal may be one of the important indicators of the global environmental changes.

References
Two Cases of Feline Panleukopenia without Prominent Diarrhea

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Keywords: cat, enteritis, panleukopenia, parvovirus infection

Introduction

Feline panleukopenia is caused by Feline panleukopenia virus (FPV), which belongs to Parovirinae. This disease is characterized by a panleukopenia, vomit, and diarrhea. FPV is highly contagious so this disease often takes an acute course with sudden death. Two cats with acute panleukocytopenia and no prominent diarrhea, negative for FPV immunomigration test, were pathologically examined in the study.

Material and Methods

One year old two adult male mix cats, kept together, died suddenly without prominent diarrhea. Hematological examination and immunomigration tests for detection of parvo viral antigen in fecal samples were conducted. After death of these 2 cats, necropsy and sample correction were performed. Tissue samples were taken from the digestive tract in addition to the liver, spleen, kidney, heart, lung and mesenteric lymph nodes. These samples were stained with haematoxylin and eosin.

Results and Discussion

Hematological examination revealed marked decrease of total white blood cell count, platelets, total protein and albumin (Table 1). FPV immunomigration test for detection of viral antigen was negative. At necropsy, swelling of the peritoneal lymph nodes, especially mesenteric lymph node, and erosive changes in the stomach, small and large intestines and icterus were observed. On histological examination, the lesions from duodenum to colon were characterized by extensive degeneration and loss of mucosal epithelium. Dilation of crypts (Fig. 1A), regeneration of mucosal epithelium and existence of numerous bacterial colonies were observed. Necrotizing enteritis with amphophilic intranuclear inclusion bodies was prominent in the colon (Fig. 1B). In lymph nodes, atrophy of lymph follicles with lymphocytic depletion was marked (Fig. 2). The bone marrow showed few megakaryocytes and bone marrow hypoplasia (Fig. 3).

FPV have an affinity and requirement for actively dividing cells. The main target tissues are the rapidly dividing cells of lymphoid tissue and the bone marrow, leading to panleukopenia, and the crypt epithelium of the intestinal mucosa, leading to enteritis. These 2 cats didn’t show prominent diarrhea and positive response for FPV immunomigration test. However, marked necrotizing enteritis with amphophilic intranuclear inclusion bodies, which are characteristic findings of FPV enteritis, were observed in addition to the atrophic findings in lymphoid tissue and the bone marrow.

Based on these clinical and pathological findings, the two cats were diagnosed as feline panleukopenia (FPV enteritis).

Table. 1 Hematological findings (Cat No.1)

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cells (x10⁹/μl)</td>
<td>1.0</td>
<td>21.0</td>
<td>4.0</td>
<td>60-150</td>
</tr>
<tr>
<td>Platelets (x10⁴/μl)</td>
<td>21.0</td>
<td>10.2</td>
<td>4.7</td>
<td>20-45</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>6.4</td>
<td>5.0</td>
<td>5.1</td>
<td>5.6-7.8</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>2.5</td>
<td>1.9</td>
<td>1.7</td>
<td>2.8-4.0</td>
</tr>
</tbody>
</table>

Fig. 1A Colon; cat No.1 dilation of crypts (arrow).
Fig. 1B Colon; cat No.1 amphophilic intranuclear inclusion body in mucosal epithelial cell (arrow).
Fig. 2 Mesenteric lymph node; cat No.1 atrophy of lymphoid follicles with lymphoid depletion.
Fig. 3 Bone marrow; cat No.2. Bone marrow hypoplasia.

References

Malignant Odontogenic Tumor in a Dog

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Keywords: dog, malignant, odontogenic tumor

Introduction

Tumors of odontogenic origin are rare in domestic animals. They often occur in young animals. They are classified according to whether they are of epithelial (ameloblastoma, amyloid-producing odontogenic tumor etc) mesenchymal (cementoma, cementifying fibroma etc) or of mixed epithelial and mesenchymal origin (ameloblastic fibroma, ameloblastic fibro-odontoma etc) (1,2). Odontogenic tumor is generally considered as benign in animals. There are a few reports on malignant odontogenic tumor in dogs (3). In this study, we describe a malignant odontogenic tumor which occurred in gingival of a dog.

Material and Methods

A 1-year-old female Labrador retriever dog had a mass on left lower jaw gingiva. The mass was surgically removed. But one month later, the mass recurred and rapidly grew. Radiologically, a tooth-like material was present in the mass. The mass showed osteolysis (Fig. 1) and infiltrated lower jaw bone.

For light microscopical examination, removed mass was fixed in 10% formalin. The tissues were routinely processed and stained with hematoxylin and eosin (HE). Immunohistochemical analysis was performed using antibodies specific for amelogenin, cytokeratin and vimentin by Labeled Streptavidin-Biotin (LSAB) method.

Results and Discussion

Histologically, the mass was composed of neoplastic spindle to ovoid cells. Most neoplastic cells showed diffuse growth pattern with fibrous stroma. They showed marked atypia and many mitotic figures. So we thought it was much undifferentiated. In addition, moderate to marked hemorrhage and necrosis of neoplastic cells were seen. These findings suggested malignancy of the tumor.

Occasionally, deposition of eosionophilic substance with calcification (Fig. 2) were observed. The neoplastic cells around them were arranged in cord, like odontogenic epithelium (Fig. 3). Immunohistochemically, the neoplastic cells and eosinophilic substance were amelogenin-positive (Fig. 4). Amelogenin is an enamel matrix protein produced by ameloblast (4). So we thought eosinophilic material was enamel and amelogenin-positive neoplastic cells were derived from ameloblast. Thus, we diagnosed this tumor as malignant odontogenic tumor.

To examine more details, immunohistochemical analysis for cytokeratin and vimentin was performed. Most of neoplastic cells were positive for vimentin (Fig. 5). Cyto-

Fig. 1  Radiograph, osteolysis of left lower jaw (arrow).

Fig. 2  Low magnification of HE stain, eosinophilic materials with calcification (arrow).

Fig. 3  High magnification of HE stain. neoplastic cells are arranged in cord (arrows).

Fig. 4  High magnification of HE stain, marked atypism and many mitotic figures (arrows) of neoplastic cells. 

Fig. 5  Immunohistochemically, some amelogenin- positive cells are found (arrows).

Fig. 6  Immunohistochemically, many vimentin-positive cells are seen.

References

Pathological Findings of “Cherry Eye” in Two Dogs

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Keywords: cherry eye, third eyelid gland, third eyelid (nictitating membrane)

Introduction

Cherry eye is the term used to refer to canine third eye lid gland prolapse. Commonly affected breeds include the Bulldog, Chihuahua, Cocker Spaniel, Beagle, Pekingese, Neapolitan Mastiff, and Basset Hound. The third eyelid gland, known as the nictitating membrane, prolapses and becomes visible. The exact mechanism is not known, however, cherry eye may be caused by a hereditary weakness in the connective tissue surrounding the gland. It is most common in puppies. It appears as a red mass in the inner corner of the eye. After gland prolapse, the eye becomes chronically inflamed and there is often a discharge. Because the gland is responsible for about 30% of the eye’s tear production, the eye can eventually suffer from dryness. Dry eye may eventually occur in 30 to 40 percent of dogs that have the gland removed. Thus, cherry eye is defined from the stand point of gross pathological finding, but not defined from the stand point of histopathological finding. In the present paper, histological findings of “Cherry eye” are reported.

Material and Methods

Two young dogs, one male, Beagle dog about 4 months old (case 1) and one female, Bulldog about 3 months old (case 2) were used. Prolapsed nictitating membranes in the right eyes of the two dogs were taken and prepared for histological examination. Four-micron-thick sections were cut, stained with hematoxylin and eosin, and examined under light microscope.

Results and Discussion

Case 1: Mild glandular hyperplasia of the third eye lid gland was observed.

Case 2: Moderate glandular hyperplasia of the third eye lid gland with interlobular fibrosis was observed.

Marked infiltration of inflammatory cells (neutrophils, macrophages and lymphocytes) at the surface of the prolapsed nictitating membrane was also observed.

Both two dogs, young and belong to particular breeds known to be commonly affected, showed “Cherry eye” with hyperplastic changes of third eyelid gland. The observed hyperplastic changes in the gland may develop secondary to the inflammation (observed in case 2). Further study is required to elucidate the mechanism of prolapse of the nictitating membrane.

References

Visceral Gout with Amyloidosis in a Humboldt Penguin (*Spheniscus humboldti*)

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Keywords: amyloidosis, Humboldt Penguin, visceral gout

Introduction

Gout is a common finding during necropsy of poultry (1). It is the result of abnormal accumulation of urates and occurs as tow distinct syndromes.

Articular gout is characterized by tophi, deposits of urates around joints, particularly those of the feet. As it has been reproduced by feeding high-protein diets, gout are suggested to result from excess production of uric acid. Defect in tubular secretion of the uric acid may be involved in the development of the disease (1).

Visceral gout, known as visceral urate deposition, is characterized by precipitation of urates in the kidneys and on serous surface of the heart, liver, mesenteries, air sacs and peritoneum. In severe cases, involvement of parenchyma of the liver and spleen may be observed. Because much urate is lost during the tissue process for histology, the lesion of urate deposits are observed as blue or pink amorphous material under microscope. Visceral urate deposition is generally due to a failure of urinary excretion. This may be due to obstruction of ureters, renal damage or dehydration. Dehydration due to water deprivation is a common cause of visceral gout in domestic poultry. Outbreaks of visceral gout in poultry have also been attributed to vitamin A deficiency, excess dietary calcium, treatment with sodium bicarbonate, and a mycotoxin, oosporein.

There are few reports on the occurrence of visceral gout in penguin. Here, we report clinical and pathological findings of the visceral gout in a Humboldt penguin (*Spheniscus humboldti*).

Material and Methods

A 16-year-old, female, Humboldt penguin died suddenly after prolonged treatment by antibiotics for the inflammatory foot lesions. Hematology and blood chemistry demonstrated increased number of white blood cells (12,000) and increased level of uric acid (17.1 mg%) and blood urea nitrogen (42.2 mg%). Calcium level was within a normal range (11.5 mg%). At necropsy, the animal was emaciated; 2.74 kg in body weight, showing one kg loss in the past one month before death. On gross examination, liver was swollen and had thickened capsule and tiny yellowish white nodules in the parenchyma (Fig. 1). Yellowish white tiny spots were also observed in the kidney. Three yellowish nodules, 1x1x1 mm, were observed in the canal of the ureter. Tissue samples from a variety of organs were taken and prepared for histological examination. Four-micron-thick sections were cut, stained with hematoxylin and eosin and examined under light microscopy.

Results and Discussion

Histological examination showed a large number of amyloid nodules consisting of amorphous pink material in the liver (Fig. 2). Tiny nodules (tophi) with bluish crystals were observed in the kidney (Fig 3). Based on these clinical and pathological findings, the animal was diagnosed as visceral gout with amyloidosis. Presence of some tiny nodules in the ureter of the bird suggests that failure of urinary excretion may be involved in the disease process. Precise mechanism of the disease entity in this penguin is to be elucidated.

References

Glaucoma Induced by Lens Luxation in the Serow: A Case Report

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Keywords: enucleation, glaucoma, intraocular pressure, lens luxation, serow

Introduction
Luxation of the lens is a result of rupture of the zonular fibers. The lens displaced in the anterior chamber obstructs the flow of aqueous humor, which likely leads to glaucoma (1). We report in the present study secondary glaucoma possibly induced by lens luxation in the serow (Capricornis sumatraensis), a relatively short-bodied, long-legged goat-antelopes in family Bovidae that is considered reserved animal of Thailand under the Thai Wildlife Protection Act and Vulnerable Animal (VU) under IUCN Red List of Threatened Species (2).

Material and Methods
A nineteen-year old, male serow at Dusit Zoo, the Zoological Park Organization, Bangkok, Thailand had had right ocular pain for one week prior to a referral to the Ophthalmology Clinic, Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University, Bangkok. Thorough ophthalmic examinations of the right eye were performed under general. Globe was slightly enlarged. The cornea was entirely edematous. Deep corneal vascularization was noticed, along with an inflammation of the conjunctiva. The mean intraocular pressure (IOP) measured with an applanation tonometer (Tono-pen XL5; Medtronic Solan, FL, USA) one week prior to the surgery was 36 mmHg on the right eye and 11 mmHg on the other. When examined with slit-lamp biomicroscope, the right lens was displaced into the ventral part of the anterior chamber.

Transpalpebral enucleation of the right eyeball was performed. An eyeball was submerged into 10% buffered formalin for routine histological process. It was embedded in paraffin wax, cut at 4 μm thickness and stained with hematoxylin & eosin.

Results and Discussion
Vacuolation of epithelial columnar cells may be intraepithelial edema that was associated with severe corneal edema. Increase number of small capillaries apparent in the superficial corneal stroma was an indication of keratitis possibly associated with an elevation of the IOP and displaced lens (3, 4). Not only collapse of the trabecular meshwork could be recognized. There was also an accumulation of red blood cells and a few inflammatory cells close to the ciliary cleft. Retina and optic nerve had not been histopathologically affected by the secondary glaucoma in this early stage.

Removal of the eyeball via transpalpebral approach was selected for better quality of life. The animal recovered well from the surgery without complications. Glaucoma in serow could be induced by lens luxation.

References
Apoptosis in Normal Bitch Mammary Tissues in Relation to Ovarian Steroid Hormones

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Keywords: apoptosis, bitch, estrous cycle, mammary gland

Introduction
Apoptosis is a key role to maintain mammary tissue homeostasis in the absence of pregnancy and lactation. Previous study on apoptosis in mice mammary tissue during estrous cycle revealed that apoptosis is regulated by estrogen and progesterone (1). However, apoptosis in bitch mammary tissues during estrous cycle have not been done before. Therefore, this study aim to investigate the apoptosis in bitch mammary tissues correlated with ovarian steroid hormones levels.

Material and Methods
Five bitches, all without mammary gland lesion were used. The estrous cycle of each bitch was determined and categorized into 6 stages (anestrus, proestrus, estrus, early diestrus, mid diestrus and late diestrus) by vaginal cytology, serum progesterone level (2). Apoptosis of bitch mammary tissues during different stages of estrous cycle were studied by using TUNEL assay.

Results and Discussion
The apoptotic bodies were found in the epithelia (both alveolar and tubular epithelium) and stromal tissue (Fig. 1). The highest percentage of apoptotic bodies in alveolar, tubular epitheliums and stromal tissue were observed at mid-diestrus when compared with the others (Table 1). This finding suggested the positive correlation between apoptotic rate of the mammary tissues and the progesterone level. This is similar to the mice mammary tissue that found high apoptotic rate during diestrus. The results of our study supported the finding that proliferative and secretory activity of mammary cells are also undergo cell death and it demonstrated that during each cycle, the mammary epithelial cells are terminally differentiated and removed by means of apoptosis.

Acknowledgements
This work was granted by Faculty of Veterinary Science, Mahidol University

Table 1 Apoptosis percentage of bitch mammary tissue

<table>
<thead>
<tr>
<th>Stage</th>
<th>Alveolar epithelium</th>
<th>Tubular epithelium</th>
<th>Stroma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anestrus</td>
<td>9.41±10.51</td>
<td>5.38±5.55</td>
<td>Negative</td>
</tr>
<tr>
<td>Proestrus</td>
<td>15.34±6.05</td>
<td>10.28±12.66</td>
<td>3.43±5.51</td>
</tr>
<tr>
<td>Estrus</td>
<td>10.49±6.15</td>
<td>6.32±5.59</td>
<td>Negative</td>
</tr>
<tr>
<td>Early diestrus</td>
<td>14.47±12.38</td>
<td>18.90±13.46</td>
<td>3.07±6.87</td>
</tr>
<tr>
<td>Mid diestrus</td>
<td>52.86±12.86</td>
<td>30.36±9.04</td>
<td>18.16±18.12</td>
</tr>
<tr>
<td>Late diestrus</td>
<td>6.75±8.28</td>
<td>2.21±2.05</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Fig. 1 Apoptotic body in alveolar epithelium (A) and tubular epithelium (B) during mid diestrus (Arrow) and alveolar epithelium (C) and tubular epithelium (D) during anestrus

References
Study of Sex Steroid Receptors in True Hermaphrodite Gilts

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Keywords: gilt, sex steroid receptor, true hermaphrodite

Introduction

True hermaphrodite is a condition that gonadal tissues of both sexes are present in an animal. Though, it was rarely found in domestic animals, the high frequency was reported in pig (1, 2). There are studies about the steroid levels in intersex female pigs and it reported the changes from the normal levels of sex hormones in these gilts (2, 3). However, the study of steroid receptors in these hermaphrodite gilts has not been done before. Therefore, the present study aims to investigate the expression of sex steroid receptors in different gonadal tissue of hermaphrodite gilts.

Material and Methods

Two hermaphrodite gilts were studied. Their genital organs were collected from the slaughter house and histologically processed. Immunohistochemistry was applied to study the expression of oestrogen receptor alpha (ER\(\alpha\)) and progesterone receptor (PR) in various genital organs of hermaphrodite gilts.

Results and Discussion

In two hermaphrodite gilts used in this study, both gilts showed ovotestis in one side of the ovary. Moreover, one gilt had shown ductus deferent-like structure at the mesometrial side of the uterus. For the results of immunohistochemistry, in most genital organs examined which were ovotestis, uterus, ductus deferent-like structure, it was shown that there was almost no expression of ER\(\alpha\) and PR in these gonadal tissues. However, in the ovarian tissue of the ovotestis, few positive ER\(\alpha\) cells could be observed in the granulosa cells of the ovarian follicles. However, as ER\(\alpha\) and PR may not be the major subtypes of steroid receptors in the ovary and testis, therefore the expression of these receptors may be restricted in these genital organs. When comparing immunostaining results of the uterus from hermaphrodite gilts to positive controls which was the uterus from normal gilt, strong staining was found in all compartments of the normal uterus. In the uterus of hermaphrodite gilts, no positive cells was found in the present study may suggest that the absence of these steroid receptors may involve with the pathological changes in these hermaphrodite gilts. In summary, since sex steroid hormones and their receptors interplay the role of regulating various reproductive physiology, the defects in reproductive functions in these gilts may be the results from the absence of these steroid receptors in gonadal tissues.

Fig. A ER\(\alpha\) positive control, uterus from normal gilts. Bar represents 100 \(\mu\)m
Fig. B ER\(\alpha\) immunostaining in the uterine tissue of hermaphrodite gilt

Fig. C ER\(\alpha\) immunostaining in the ovarian tissue of ovotestis
Fig. D ER\(\alpha\) immunostaining in the testicular tissue of ovotestis

References

Study of Doxorubicin Chemotherapy for Malignant Canine Mammary Gland Tumors

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Keywords: canine, doxorubicin, malignant, mammary gland tumor

Introduction

Mammary gland tumor is the high incident disease which could be found secondly after skin tumor in the dogs. In general, mammary gland tumor could be found more frequently in bitch than male dog (1) and it accounts to 25-50 percent of various kinds of tumor found in the bitch (2, 3). Moreover, with in these numbers, there is a fifty percent tendency of the tumors to become malignant. Until present, the classical treatment which is most effective is the surgical removal of these tumors, but there still be high risk of surgical complication. Chemotherapy is an alternative treatment that are able to apply in bitch which prone to have surgical complication. Therefore, the aim of this present study is to investigate the effect of doxorubicin chemotherapy on canine mammary tumors.

Material and Methods

Fifteen dogs with mammary gland tumors were used in the present study. The chemotherapy with doxorubicin in a dosage of 30 mg/m² was administered to these dogs intravenously. In order to study the effect of doxorubicin on the mammary tumor, the diameter of tumor mass and histopathological changes were determined before and after the treatment.

Results and Discussion

The results showed that diameters before and after treatment were not statistical different. After the treatment with doxorubicin, the results demonstrated partial remission (PR) in 2 dogs (13.33 %), stable disease (SD) in 11 dogs (73.34 %) and progressive disease (PD) in 2 dogs (13.33%). Histopathological changes showed more collagen and fibroblast cells in 3 dogs. It can be concluded that doxorubicin 30 mg/m² may have some effects on the growth or stabilize the progress of the tumor and that doxorubicin alone is not suitable for treatment canine mammary gland tumor. Moreover, the results of this study may suggest the surgical removal of the tumor in addition with doxorubicin therapy especially in the early stage of the mammary gland tumor.

Fig. A1 Tubular adenocarcinoma, simple type: before doxorubicin chemotherapy.
Fig. A2 Tubular adenocarcinoma, simple type: after doxorubicin chemotherapy

Fig. B1 Tubular adenocarcinoma, simple type: before doxorubicin chemotherapy.
Fig. B2 Tubular adenocarcinoma: after doxorubicin chemotherapy.

Fig. C1 Spindle cell carcinoma: before doxorubicin chemotherapy.
Fig. C2 Spindle cell carcinoma: after doxorubicin chemotherapy.

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

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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), sw/Chachoengsao/NIAH-03/03(H3N2) and sw/Ratchaburi/NIAH874/05 (H3N2); these four isolates belong to classical swine lineage. From phylogenetic analysis of the NP nucleotide, cluster of classical swine indicates closely related with human lineages more than avian lineages at amino acid position 33, 100 and 136. 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 33°</th>
<th>Amino acid position 100°</th>
<th>Amino acid position 136°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>I</td>
<td>V</td>
<td>I</td>
</tr>
<tr>
<td>Avian</td>
<td>V</td>
<td>R</td>
<td>L</td>
</tr>
<tr>
<td>Sw/NIAH586-4/05(H3N2)</td>
<td>I</td>
<td>V</td>
<td>I</td>
</tr>
<tr>
<td>Sw/NIAH586/05(H3N2)</td>
<td>I</td>
<td>V</td>
<td>I</td>
</tr>
<tr>
<td>Sw/NIAH-03/03(H3N2)</td>
<td>I</td>
<td>V</td>
<td>I</td>
</tr>
<tr>
<td>Sw/NIAH874/05(H3N2)</td>
<td>I</td>
<td>V</td>
<td>I</td>
</tr>
</tbody>
</table>

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

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

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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 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 milk &amp; dairy products</th>
<th>Desserts</th>
<th>Dog sticks</th>
<th>Human snacks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mast cell tumor</td>
<td>92.11</td>
<td>73.68</td>
<td>86.84</td>
<td>56.58</td>
</tr>
<tr>
<td>Malignant mesenchymal tumor</td>
<td>71.74</td>
<td>65.22</td>
<td>63.04</td>
<td>58.70</td>
</tr>
<tr>
<td>Lymphoid tumor</td>
<td>83.78</td>
<td>75.68</td>
<td>89.19</td>
<td>67.57</td>
</tr>
<tr>
<td>Malignant epithelial tumor</td>
<td>75.76</td>
<td>69.70</td>
<td>78.79</td>
<td>36.36</td>
</tr>
<tr>
<td>Melanoma</td>
<td>80.80</td>
<td>80.92</td>
<td>82.61</td>
<td>39.13</td>
</tr>
<tr>
<td>Mammary tumor</td>
<td>78.26</td>
<td>69.57</td>
<td>82.61</td>
<td>39.13</td>
</tr>
<tr>
<td>Others</td>
<td>90.90</td>
<td>70.70</td>
<td>70.50</td>
<td>50.60</td>
</tr>
</tbody>
</table>

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

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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 circo virus 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

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References

Study of Aquaporin 1 (AQP1) on Acute kidney injury

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

Introduction

Acute kidney injury (AKI), also has 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

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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 intratumorouso 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*)

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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 deppresses, 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 hemotoma 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 in vitro Infection of Canine Glia

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

Introduction

Canine distemper (CD) is a highly contagious and immunosuppressive 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 (p75NTR)-positive brain cells has been recently shown as a preferential infected cells following CDV inoculation 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% CO2, water-saturated atmosphere) (4). Cells from passages 5-7 were seeded in 6-well PLL-coated plates (NuncTM) (1.6x105 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® Europe, Amsterdam, the Netherlands). The sequence of primer pairs were used as follows:

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

SLAM
F: 5’-TGG AAA GCA GGA GGG AAA ATG A-3’ (280 bp)
R: 5’-TGA GGG CCG AGG CTG AGG TG-3’

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

p75NTR
F: 5’-TGA GTG CTG CAA AGC CTG CAA-3’ (229 bp)
R: 5’-TCT CAT CCT GGT AGT AGC CGT-3’

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 p75NTR 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 p75NTR did not differ 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 in vitro. In addition, the differential expression of CD9 and p75NTR 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

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References

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

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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 subsequence 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 seen. 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 β-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 β-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).  

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**References**  
Systemic Spirochidiasis in a Green Sea Turtles (*Chelonia mydas*)

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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 Australa. 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 granulomatus 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 granulomatus 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

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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. Vacuolar 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.

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References

Renal Myxozoanosis in a Soft-shell Turtle

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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 melanomacrophages 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 (2-3 μm) 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.

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References

Microsporidia Infection in Swordtail fish, *Xiphophorus helleri*

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**Keywords:** microsporidia, swordtail fish

**Introduction**

Thailand produces and exports a lot of ornamental fish every year. The fish were exported to many countries include 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 included 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.

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**References**

Acute Oral Toxicity Test of Colloidal Silver Nanoparticles

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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, hiliar 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

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References

Acute Dermal Toxicity Test of Colloidal Silver Nanoparticles

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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 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.

Fig. 1 Skin appearance of 100,000 ppm group at 0 hr post-exposure (A) and 24 hr post-exposure after residue removing (B)

Fig. 2 Skin biopsy from 100,000 ppm group, Ag-NPs cover on the keratin layer (arrow) H&E, x390.

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

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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

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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

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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 immunoactivity 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>
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<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>Neurofibroma</td>
<td>1</td>
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</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

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References

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

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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

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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 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 metropolitans using the Kiels 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.
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

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<th>Table 1</th>
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<tr>
<td>Anatomical classification</td>
<td>Number of cases</td>
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<tr>
<td>Multicentric</td>
<td>23 (57.5%)</td>
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<tr>
<td>Mediastinal</td>
<td>1 (2.5%)</td>
</tr>
<tr>
<td>Alimentary</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>Extranodal</td>
<td>8 (20%)</td>
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<tr>
<td>Cutaneous</td>
<td>6 (15%)</td>
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<table>
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<th>Table 2</th>
<th>The Histological and Immunophenotyping classification according to the Kiel’s classification</th>
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<tr>
<td>B cell</td>
<td>T cell</td>
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<tr>
<td>No. of cases</td>
<td>No. of cases</td>
</tr>
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<td>-----------------------------------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Low-grade malignancy</td>
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<td>Malignancy</td>
<td>Malignancy</td>
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<tr>
<td>Small cell</td>
<td>Small cell</td>
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<tr>
<td>- Lymphocytic</td>
<td>- Lymphocytic</td>
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<tr>
<td>- Lymphoplasmacytic</td>
<td>- Lymphoplasmacytic</td>
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<tr>
<td>- Prolymphocytic</td>
<td>- Prolymphocytic</td>
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<td>- Centrocytic</td>
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<td>- Centrocystic/ Centrocytic</td>
<td>- Centrocystic/ Centrocytic</td>
</tr>
<tr>
<td>- Macronucleolated</td>
<td>- Macronucleolated</td>
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<tr>
<td>- Medium-sized cell</td>
<td>- Medium-sized cell</td>
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<tr>
<td>- Immunoblastic</td>
<td>- Immunoblastic</td>
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<tr>
<td>- Plasmacytoid</td>
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<td>- Lymphoblastic</td>
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**In Vitro Antiproliferation Activity of Temulawak (Curcuma xanthorriza Roxb.) Ethanol Extract on YAC-1 and HeLa Tumor Derived Cell Lines**

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**Keywords:** antiproliferation, Curcuma xanthorriza, 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. xanthorriza* known as “Temulawak” in Indonesian language is one of 7,000 Indonesia’s medicinal plants out of 30,000 plants found in Indonesia; this number accounted as a 90% of Asia’s medicinal plants (1) Ethanol extracts of the root of *C. xanthorriza* (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. xanthorriza* on tumor-derived cell lines in order to find the anti-tumor drugs for medical purposes both in human and animal.

**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. xanthorriza* 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.

**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.

**Fig. 1** The plant with flower (left) and root (right) of *C. xanthorriza* (source: www.geocities.com)

**Fig. 2** Dead tumor cells on the hemacytometer counter. Trypan blue dye. (bar = 40 μm).

The degree of this activity was varied in each cell lines. The highest anti-proliferation activity of *C. xanthorriza* 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-inflammation, anti-oxidant and hypocholestemic (4). The other components in C. xanthorriza were consists of camphor, mirsen, xanthorizol, \(\beta\)-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 Bel-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-transfarse 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 (Bel-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.
7. Thangapazham et al., 2006. AAPS J. 8(3): 443-449
The Supplementation of *Andrograpis panicula* in the Feed of Lactating Sows Reduce Pre-weaning Mortality and Increase Number of Piglets at Weaning

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**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 antityphoid, antifungal, antihepatotoxic, antibiotic, antimalarial, antihepatitic, antithrombogenic, antiinflammatory, anti-snake venom and antipyretic properties (3). It is also generally use 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: \( \text{ADG (g/day)} = \frac{[(\text{Average weaning weight-average birth weight})/\text{lactation length}]}{1,000} \). Pre-weaning mortality rate (PWM) was calculated using the following formula: \( \text{PWM (%) = } \frac{[(\text{BA-wean})/\text{BA}] \times 100} \). 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.

![Frequency distribution of the body weight at weaning (kg) of the piglets](image)

**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$^1$</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&quot;&quot;</td>
</tr>
<tr>
<td>Weaning weight (kg)</td>
<td>6.6±1.1</td>
<td>6.4±1.0</td>
<td>-0.2&quot;</td>
</tr>
<tr>
<td>Litter weight gain (kg)</td>
<td>44.7±13.4</td>
<td>48.1±14.4</td>
<td>+3.3&quot;&quot;</td>
</tr>
<tr>
<td>PWM$^3$ (%)</td>
<td>14.6</td>
<td>2.2</td>
<td>-10.4&quot;&quot;</td>
</tr>
</tbody>
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1BA, number of piglets born alive/litter;  
2Wean, number of piglets at weaning;  
3PWM, pre-weaning mortality, "", $p<0.001$, "", $p<0.01$

![Number of piglets at weaning (least-squared means) in control and treatment groups by farrowing house (A, C and G); a,b $p<0.05$](image)

![Litter weight gains (kg) (least-squared means) in control and treatment groups by farrowing house (A, C and G); a,b $p<0.05$](image)

**References**

Diagnostic Cytology in Veterinary Clinical Medicine

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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
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 supplicative, 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 supplicative (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 background 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

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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; and genotype F is identified mostly in the American populations of Central America and South America; and genotype H is confined to the Amerindian populations of South America; and 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 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
Suppression of Rabies Virus Propagation in Mice Brain by Intracerebral Immunization of Inactivated Virus

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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 elicit 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

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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 1x10⁶ 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

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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 likely 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 internal organs. For dermal lesions forget hyperplasia and normal structure, you are aspirating a lump and it is not normal. Decide this: 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.

KEY = the more variable the cells and nuclei, the more likely it is neoplasia; the more uniform they are = normal, hyperplasia, or adenoma.

Determination 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 criterion 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!

**CYTOLIGIC 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.

**KEY**

4. High nuclei-cytoplasmic ratio-large nuclei.
5. Nuclear abnormalities
   - Multiple nuclei, varying numbers
   - Large
   - Variation in shape and size of nuclei
   - Lobation, 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

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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 subsampling 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) Clinician’s 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 clinician’s 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 pathologist’s 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

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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 neuraminidase 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. 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 results, 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 ted 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
duck/Vietnam/209/05. 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 day14 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

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**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

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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*)

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**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 centrilobular hemorrhage and necrosis along with moderate perilobular fatty change in all lobules. Small numbers of neutrophils along with variable amounts of brown pigment and cell debris are present in the centrilobular 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, ferrie (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*)


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**Keywords:** horse, Mongolain, septicmia

**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 cocccobacilli 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 a major cause of death in critically ill foals, regardless of their primary disease. Affected foals may be abnormal at birth or they may appear clinically normal initially and then develop signs of illness during the first few hours or days after birth.

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 prevalence 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 septicmic 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 tinged with blood, rather than frankly purulent as with *Streptococcus zooepidemicus and Salmonella spp*. *E. coli* septicmia 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 catarrah 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 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**

Cryptococciosis in a Cat

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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 neoformans, 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, lymphoadenopathy, 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

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Keywords: dog, lymphoma, mycobactriosis

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-phochromogen 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 histiocytoma. 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

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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 heterogenous 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 basophilc 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

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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

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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 fibrohistiocytic 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, showned 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