

***7<sup>th</sup> Asian Meeting on Zoo and Wildlife Medicine/Conservation  
“One Health in Asia Pacific”***

***DATE: 14 –17<sup>th</sup> October; 2014***

***VENUE: Vietnam National University of Agriculture, Tam Dao  
National Park and Ha Long City***

***HOST ORGANIZATIONS***

- *Asian Society of Zoo and Wildlife Medicine (ASZWM)*
- *Asian College of Conservation Medicine (ACCM)*
- *Vietnam National University of Agriculture (VNUA)*
- *Institute of Ecology and Biological Resources (IEBR), Vietnam Academy of Sciences*
- *Hanoi Zoo*
- *Vietnam Veterinary Association*
- *Vietnam Bear Rescue Centre, Animals Asia Foundation*
- *Federation of Asian Veterinary Association (FAVA)*
- *Asian Association of Veterinary Schools (AAVS)*

***Supports from Gifu University, SNU, FAVA, Taipei Zoo, JSZWM, KSZWM***

***Organization committee:***

- *Asian Society of Zoo and Wildlife Medicine (ASZWM)*
- *Vietnam National University of Agriculture (VNUA), Vietnam*
- *Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology (IEBR), Hanoi, Vietnam*
- *Hanoi Zoo, Vietnam*
- *Vietnam Veterinary Association, Vietnam*

***Organization committee members:***

***Vietnam***

- *TRINH Dinh Thau, Dean, Faculty of Veterinary Medicine, Vietnam National University of Agriculture, Vietnam*
- *LE Xuan Canh, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, Hanoi, Vietnam*
- *DANG Gia Tung, President, Hanoi Zoo, Hanoi, Vietnam*
- *Chairman, NGUYEN Thi Lan, Vice Dean, Faculty of Veterinary Medicine, Vietnam National University of Agriculture, Vietnam*
- *NGUYEN Truong Son (Secretary) and Pham Ngoc Doanh, Institute of Ecology and Biological Resources, Hanoi, and Nguyen Van Giap (Secretary) from VNUA*
- *Joost Philippa, Senior Veterinarian, Animals Asia, Vietnam Bear Rescue Centre, Tam Dao National Park*

***ASZWM***

- *Tokuma Yanai, Senior Board, Professor, Gifu University, Japan*
- *Junpei Kimura, Secretary General ASZWM, Professor, Seoul National University, Korea*
- *Nabin Rayamajhi, Vice Secretary General ASZWM, Asst. Professor, Patan Academy of Health Science, Nepal*
- *Toshio Mizoguchi, Project leader, Fukushima Prefectural Government Natural Conservation Division, Fukushima Wildlife Rehabilitation Center, Japan*
- *Alice Lau Ching Ching, College of Veterinary Medicine, Seoul National University, Korea*
- *Sunmin Kim, College of Veterinary Medicine, Seoul National University, Korea*
- *Nobuhide Kido, Nogeyama Zoological Gardens, Japan*

## **New Challenges by Asian Society of Zoo and Wildlife Medicine (ASZWM) for “One Health in Asia Pacific**

Tokuma Yanai<sup>1</sup> and Junpei Kimura<sup>2</sup>  
Senior Board Member<sup>1</sup> and Secretary General<sup>2</sup> of ASZWM

Since the first meeting at Kasetsart Univ. in 2005, ASZWM has been holding annual meetings, to establish an Asian network for conservation medicine, with the goal of “One Health in Asia Pacific.” These annual meetings have been hosted by various veterinary colleges, such as Chulalongkorn Univ. (2006), National Taiwan Univ. (2007), Bogor Agricultural Univ./ IVA (2008), Seoul National Univ. (2009), Univ. of Putra Malaysia/MVA (2010), Tribhuvan Univ /NVA (2011), Mahidol Univ. (2012) and Singapore Zoo/SVA (2013); some of these meetings were in collaboration with local veterinary associations. In every meeting there were many student participants who were interested in wildlife conservation and “One Health”, and who gave oral or poster presentations, as well as student volunteers from the host universities.

This time, as a new challenge, ASZWM is arranging an educational pre-congress workshop for local and overseas students as well as local zoo veterinarians. ASZWM and Vietnam National University of Agriculture is arranging this pre-congress workshop with support from the Federation of Asian Veterinary Association, Gifu University and OIE. We believe these meetings can provide high-quality one-health education, especially in eco-system conservation and veterinary science, to local students.

Based on previous open discussions in Singapore on “How to collaborate with Wildlife Diseases Association (WDA) to establish a wildlife diseases surveillance system in Asia by ASZWM”, the ASZWM board joined the 63th WDA meeting and agreed to start each discussion for further collaboration. Future collaborations between ASZWM and WDA will mutually benefit the wildlife diseases surveillance, especially the exchange of information on infectious diseases. As a first step, the ASZWM board has arranged the ASZWM-WDA joint session for “Wildlife Diseases Surveillance in Asia” in this meeting.

From 2015, ASZWM will be renamed “Asian Society of Conservation Medicine (ASCM)” to promote collaboration with ecology departments and human medicine. To promote the “One Health” concept in Asian wildlife conservation networking, the ASZWM board has continued discussions since its meeting in Singapore, and approved the change of name of our society from Asian Society of Zoo and Wildlife Medicine (ASZWM) to Asian Society of Conservation Medicine (ASCM) in this 7th meeting in Vietnam. This change of name will encourage the participation of dedicated people from ecology, human medicine and biology who are in charge of conservation of eco-system and wildlife.

As for our diploma system, the Asian College of Conservation Medicine (ACCM), established in 2012, we currently have 25 diploma members and formed an organization to sustain the Asian Society of Conservation Medicine. The college will discuss our strategy and propose various ideas for the development of the Asian Society of Conservation Medicine.

In 2015, ten years will have passed since our first Asian Meeting of Zoo and Wildlife Medicine, only one meeting for conservation medicine in Asia, which started in Kasetsart University in Bangkok in 2005. Let us get together at the annual meeting of ASCM 2015 (in Yangon Zoo, Myanmar) to celebrate our 10th year anniversary.

Thank you again for your cooperation.



“A discussion about collaboration with WDA in Singapore 2013”



“Award of diploma at the ASZWM meeting in Singapore”

***TITLE******PAGE*****14<sup>th</sup> October****Pre-Congress Workshop at Vietnam National University of Agriculture (Hanoi)****0900-1200 “The Significance of Zoo and Wildlife Medicine (Conservation Medicine) in Asia”**

Chaired by Dr. Junpei Kimura and Dr. Nabin Rayamajhi

**OPENING REMARKS**

Dean of Faculty of Veterinary Medicine, VNUA

Dr.Achariya Sailasuta, FAVA Secretary General

**LECTURES****1. Importance of Wildlife Medicine**

Prof.Toshio Tsubota---Hokkaido University, Japan

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**2. Introduction of Zoo Medicine**

Dr.Paolo Martelli, Ocean Park Corporation, HongKong

**3. Application of Wildlife Medicine for the Human Society**

Dr.Toshio Mizoguchi, Fukushima Wildlife Rehabilitation Center, Japan

**CLOSING REMARKS**

The Role and Activities of OIE relevant to wildlife.

Dr. Tikiri Priyantha Wijayathilaka, OIE Regional Project Coordinator

Dr.Pam Whiteley, WDA member

12:30-13:30 Lunch

1400- Move to Tam Dao National Park

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1700-1800 Registration at Green World Hotel (Tam Dao National Park)

1830 Award ceremony for ACCM diploma welcome party and Welcome Dinner at Green World Hotel (Tam Dao National Park)  
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**15<sup>th</sup> October****0900-1000 OPENING SESSION (Room “Pangolin”)**

Chaired by Dr. Junpei Kimura and Dr. Nabin Rayamajhi

0900-0920 Greetings

Prof. Dr. Tran Duc Vien, President of VNUA.

Dang Gia Tung, President of Hanoi Zoo.

Prof. Dr. Dau Ngoc Hao, President of VVA.

Prof. Tokuma Yanai, Senior board of ASZWM.

**01.** 0920-0940 Wildlife Medicine Education and Research of Faculty of Veterinary Medicine, Vietnam National University of Agriculture: Achievement and Future Perspectives

Dr. Ban Tiep Nguyen, Vietnam National University of Agriculture

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0940-0955 Nature of Tam Dao.

Dr. Joost Philippa, Animals Asia Foundation

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**1000-1150 SPECIAL SESSION: “What is “Conservation Medicine”? A road to “One health”” (Room “Pangolin”)**

Chaired by Dr. Junpei Kimura and Dr. Nabin Rayamajhi

1000-1010 The Goal of This Session

Dr. Tokuma Yanai, ASZWM Senior Board, Japan

**02.** 1010-1030 General Concept for Conservation Medicine

Dr. Pam Whiteley, Melbourne University, Australia

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**03.** 1030-1100 A History of Infectious Diseases and Civilization: Conquest or Co-existence?

Dr. Taro Yamamoto, Institute of Tropical Medicine, Nagasaki University, Japan

10

**04.** 1100-1130 Conservation Medicine from Ecology –A Case Study on Behavioral Ecology of Japanese Black Bears

Dr. Koji Yamazaki, Ibaraki Nature Museum, Japan

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1130-1140 General discussion for “Asian Society for Conservation Medicine”.

Dr. Junpei Kimura, ASZWM Secretary General, Korea

**STREAM 1****1330-1600: WELFARE, MANAGEMENT and CONSERVATION (Room “Pangolin”)**

Chaired by Dr. Na Ki Jeong, Dr.Muhammad Agil, Dr Paolo Martelli, Dr Koichi Murata, and Dr. Nguyen Quang Truong

<b>O5.</b> 1330-1345 Experimental Translocation Project of Five Captive-born Amur Leopard Cats ( <i>Prionailurus bengalensis euptilurus</i> ) at Seoul Zoo, in Ansan Wetland Park Area. Dr. Kyung Yeon Eo	10
<b>O6.</b> 1345-1400 The Follow up Survey of the Released Raccoon Dog ( <i>Nystereutes procyonoides</i> ) by the Wildlife Rescue Project. Dr. Nobuhide Kido	11
<b>O7.</b> 1400-1415 Species Identification for a Rescued Wolf with Mitochondria DNA Control Region. Dr. Soh-won Bae	11
<b>O8.</b> 1415-1430 Reproductive Behaviour of Caspian Red Deer ( <i>Cervus elaphus maral</i> ) in Wildlife Refuge of Semeskande, Sari. Dr. Behrang Ekrami	12
<b>O9.</b> 1430-1445 Orangutan Veterinary Advisory Group (OVAD), an International Vet Network to Safe Orang-utans. Dr. Fransiska Sulisty	14
1445-1500 Break time	
<b>O10.</b> 1500-1515 Early Pregnancy Diagnosis in the White African Rhino using Ultrasound in Taman Safari Indonesia – Bogor. Dr. Muhammad Agil	14
<b>O11.</b> 1515-1530 Diagnosis and Management of Gastric Foreign Body Obstruction Ingestion in Penguin in a Zoological Collection. Dr. Lee Fong Khong	14
<b>O12.</b> 1530-1545 Eclipse Plumage as One of the Characteristic in Identification of Red Jungle Fowls ( <i>Gallus gallus</i> ). Dr. Syahar Amir A. Gani	15
<b>O13.</b> 1545-1600 Herpetological Exploration in Karst Forests of Laos. Dr. Nguyen Quang Truong	15
<b>STREAM 1</b>	
<b>1700-1830: ZOO AND AQUARIUM VET NETWORKING (Room “Pangolin”)</b>	
Chaired by Dr. Paolo Martelli	
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<b>STREAM 2</b>	
<b>1330-1515: PATHOLOGY (Room “Saola”)</b>	
Chaired by Dr. Wiji Banlunara, Dr. Chian-Ren Jeng, Dr. Acharya Sailasuta and Dr. Pham Ngoc Doanh	
<b>O14.</b> 1330-1345 Intestinal Mucinous Adenocarcinoma in a Clouded Leopard ( <i>Neofelis nebulosa</i> ). Dr. Chanokchon Sethawongsin	16
<b>O15.</b> 1345-1400 Pathology and Molecular Characterization of Feline Parvovirus Isolated from Small Indian Civets ( <i>Viverricula indica</i> ) in Thailand. Dr. Surangkanang Chaiyasak	16
<b>O16.</b> 1400-1415 Reptilian Paramyxovirus in Captive Boidae and Pythonidae. Dr. Piyaporn Kongmakee	17
<b>O17.</b> 1415-1430 Metabolic Bone Disease in a Veiled Chameleon ( <i>Chamaeleo calyptatus</i> ). Dr. Benchaphorn Limcharoen	18
<b>O18.</b> 1430-1445 Causes of Deaths in Scimitar-horned Oryx ( <i>Oryx dammah</i> ) in the UK. Zoological Collections. Dr. Manjula Jayasinghe	19
<b>O19.</b> 1445-1500 Pathological and Immunohistochemical Findings of Avian Encephalomyelitis Virus Infection in Captive Emu, Ostrich and American Flamingo. Dr. Mohie Haridy	20
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<b>1500-1530 Break time</b>	
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<b>1530-1645</b>	
<b>ASIAN ELEPHANT (Room “Saola”)</b>	
Chaired by Dr. Sarad Paudel, Dr. Chatchote Thitaram	
<b>O20.</b> 1530-1545 The Effect of Platelet Concentrate Gel on Wound Healing in Asian Elephant ( <i>Elephas maximus</i> ). Dr. Pakkanut Bansiddhi	20
<b>O21.</b> 1545-1600 Immune Responses on Different Dosages of Tetanus Toxoid in Captive Asian Elephant ( <i>Elephas maximus</i> ); Preliminary Study. Dr. Chaleamchat Somgrid	21
<b>O22.</b> 1600-1615 Case Report: Cecal Trocarization for Gas Decompressing in Asian Elephant ( <i>Elephas maximus</i> ). Dr. Thittaya Janyamethakul	22
<b>O23.</b> 1615-1630 Genetic Relatedness and Behaviours of Releasing Asian Elephants ( <i>Elephas maximus</i> ) in the Elephant Reintroduction Project. Dr. Chatchote Thitaram	23
<b>O24.</b> 1630-1645 Interferon-gamma Release Assay (IGRA) for the Diagnosis of Tuberculosis in Asian Elephant ( <i>Elephas maximus</i> ). Dr. Sarad Paudel	24
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<b>1830 Dinner ---</b>	

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## 16th October

### 0830-1020: ASZWM-WDA JOINT SESSION FOR ASIAN WILDLIFE DISEASES SURVEILLANCE: Rabies in Wild Animals (Room “Pangolin”)

Chaired by Dr. Akio Yamada, Dr. Tokuma Yanai

- Q25.** 0830-0845 A Collaboration between ASZWM and WDA for Wildlife Diseases Surveillance in Asia; a report of 69th Annual Meeting of WDA in Albuquerque, USA 24  
Dr. Nabin Rayamajhi
- Q26.** 0845-0905 Discovery of Rabies in a Wild Carnivore in Taiwan. 25  
Dr. Chian-Ren Jeng
- Q27.** 0905-0920 The Zombie Ferret-badger: Epidemiology and Impact of Rabies on Ferret-badger Population in the Coastal mountain Range, a Hotspot of Enzootic Area in Taiwan. 25  
Dr. Chen-Chih Chen
- Q28.** 0920-0935 Rabies Control and Vaccine Development for Wildlife in Korea. 26  
Dr. Ha-Hyun Kim
- Q29.** 0935-0950 Status of Oral Rabies Vaccination in Wild Animals in the Seoul, Metropolitan Area, Korea. 26  
Dr. Hyunkyung Jang
- Q30.** 0950-1005 Rabies in an Infant Orangutan (*Pongo pygmaeus*) at Borneo Orangutan Survival Foundation (BOSF) Nyaru Menteng. 26  
Dr. Agus Fahrani
- Q31.** 1005-1020 The Role of Animal Welfare in the Health Husbandry and Conservation of Zoo Species. 27  
Dr. Heather Bacon, University of Edinburgh – Animals Asia

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### 1020-1035 Break time

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### 1035-1205: INFECTIOUS DISEASE (Room “Pangolin”)

Chaired by Dr. Manabu Onuma, Dr. Parntep Ratanakorn

- Q32.** 1035-1050 Opportunistic and Pathogenic Mycobacteria: Importance and Systematic Surveillance of Mycobacteriosis based on Pathological Diagnosis. 27  
Dr. Takayuki Wada
- Q33.** 1050-1105 Monitoring of Outdoor and Zoonotic Infectious Diseases by using Hunting Dogs in Japan: Usefulness of Hunting Dogs as a Sentry. 28  
Dr. Tokuma Yanai
- Q34.** 1105-1120 Fatal Canine Distemper Virus Infection in Common Palm Civets (*Paradoxurus hermaphroditus*). 28  
Dr. Paisin Lekcharoen
- Q35.** 1120-1135 A Relapsing Fever Group Borrelia sp. similar to Borrelia ionestari found among Wild Sika Deer (*Cervus nippon yezoensis*) and Haemophysalis spp. Ticks in Hokkaido, Japan. 29  
Dr. KyungLee Lee
- Q36.** 1135-1150 Surveillance of Avian Influenza Virus in a Wild Bird Trade in Hanoi during Spring Migration Season 2014. 30  
Dr. Le Manh Hung
- Q37.** 1150-1205 Case Report of a Dyspneic Red-billed Hornbill Parasitized by Cyclocoelid Trematodes in Jurong Bird Park, Singapore. 30  
Dr. Mitsuhiro Asakawa

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### 1205 -1345 Lunch time

1210 1<sup>st</sup> bus to bear center (Lunch packed)

1300 2<sup>nd</sup> bus to bear center (Lunch at Green Hotel)

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### STREAM 1

### 1345-1630 MARINE MAMMALS (Room “Pangolin”)

Chaired by Dr. Yuko Tajima, Dr. Tadasu Yamada

- Q38.** 1345-1405 Cetaceans from Vietnamese Waters: A View from the Outside. 31  
Dr. Robert L. Brownell, Jr.
- Q39.** 1405-1425 Some Information about Marine Mammals in Vietnam. 31  
Dr. Pham Van Chien
- Q40.** 1425-1455 Family Ziphiidae in Asia - Two Case Studies on Population Genetics of the Baird's Beaked Whale *Berardius bairdii* and Stejneger's Beaked Whale *Mesoplodon stejnegeri* around Japan. 32  
Dr. Shino Kitamura
- Q41.** 1555-1615 Chronological, Spatial and Age Distribution of Finless Porpoises (*Neophocaena phocaenoides*) in Taiwanese Waters Based on Stranding, Bycaught and Landbased Sighting Surveys. 32  
Dr. Chiou- Ju Yao

<b>042.</b> 1515-1535 First Record of Ginkgo-toothed Beaked Whale ( <i>Mesoplodon ginkgodens</i> ) stranded in Korea. Dr. Sunmin Kim	32
1535- General discussion	
<b>1340-1800: BEAR (Tam Dao Bear Rescue Center)</b>	
Chaired by Dr. Toshio Tsubota, Dr. Joost Philippa	
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<b>044.</b> 1355-1405 Management of Rescued Bears from Bear Bile Farms at Animals Asia Sanctuary in Vietnam. Annemarie Weegenaar	33
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<b>1900---Closing ceremony (Award ceremony, Announcement of next meeting, Dinner)</b>	
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## Wildlife Medicine Education and Research of Faculty of Veterinary Medicine: Achievements and Future Perspectives

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### Abstract

Faculty of Veterinary Medicine (FVM) of Vietnam National University of Agriculture is the only independent veterinary faculty in Vietnam with 58 years of veterinary education. For more than 5 decades, food animals have been the main objectives of teaching and research. It is agreed that responsibility of veterinarians is protecting the animal and human health and that veterinarians are involved in wildlife preservation and conservation. Role of wild animals in disease control together with wildlife farming in Vietnam have raised the attention in veterinary teaching and research. Vietnamese veterinary students should be aware of the global trade of wild animals, emerging infectious diseases and environmental threats; their roles in the protection of wildlife as well as opportunities and challenges in wildlife and wildlife farm health.

To increase contribution of FVM in the field of wildlife medicine and conservation, animal objectives have been diversified in teaching and research, from fish to mammals, both wild and captured animals including wild animals in farms. For wildlife – oriented approach, incorporation of wild animal objectives into various subjects of traditional curriculum has been made. Zoonosis and Wild Animal Medicine are now adopted as two subjects in new curriculum and Animal Welfare is also accepted as a selective subject (wild animal welfare is one of the main concerns). The syllabus design includes lectures, laboratories and field studies. Wild animal researches, from fundamental biology to preventive medicine have been enhanced by the faculty and publications in national and international journals have been made. The achievements have been generated as the outcomes of domestic and international collaborations.

To become a center of wildlife medicine education and research of the country, we are looking forward to collaborating with and obtaining supports from our present and putative collaborators.

Keywords: Vietnam National University of Agriculture, Wild animal research, Wildlife medicine education

### SPECIAL SESSION: “What is “Conservation Medicine”? A road to “One health””

#### General Concept for Conservation Medicine

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Conservation Medicine is closely linked with One Health and EcoHealth and arose partly from Conservation Biology. Professors Andrew P Dobson and Robert M May wrote the chapter on ‘Disease and Conservation’ in Conservation Biology (Soule, 1986) and there are several papers on Conservation Medicine in the journal Conservation Biology. In 1992 Boyce, Yuill, Homan and Jessup discussed ‘A role for veterinarians in wildlife health and conservation biology’ in the Journal of the American Veterinary Medical Association.

Conservation Medicine, One Health and EcoHealth are all concerned with the essential links between and transdisciplinary approaches to the health of ecosystems, biodiversity (including wildlife), domestic animals and humans. There is an urgent need for better understanding and management of anthropogenic impacts. There is recognition of complexity, uncertainty and the need for multidisciplinary, integrative and long-term approaches to improve health on our planet.

Post graduate Conservation Medicine programs have been developed at Murdoch University Western Australia, Tufts University USA, The University of Edinburgh UK, Massey University New Zealand and elsewhere with other programs at the Royal Veterinary College London UK, the University of California Davis, and with the Canadian Cooperative Wildlife Health Center, based at their veterinary colleges. Information is available on their websites. However, it is important to introduce conservation biology, ecohealth and one health in undergraduate curricula, ideally with students from different disciplines working together in teams investigating real world problems. EcoHealth Supplement 1 2004 published papers from a two day workshop on teaching ecosystem health in medical and veterinary curricula. In 2009 the World Organisation for Animal Health (OIE) published ‘Veterinary education for global animal and public health’ (Walsh, 2009) including two papers on ‘Essential veterinary education in conservation medicine and ecosystem health’ and ‘Essential veterinary education in zoological and wildlife medicine’ as Scientific and Technical Review by Aguirre and Gomez, and Aguirre. A Alonso Aguirre et al also published text books: Conservation medicine: ecological health in practice in 2004 and New directions in conservation medicine in 2012.

Veterinary scientists can contribute significantly to improve local and global health because of their education in clinical medicine, pathogenesis, epidemiology etc and awareness of molecular, individual animal, population and ecosystem perspectives. However, we will be much more productive when collaborating with colleagues from ecology and environment, human medicine, toxicology, climate science, mapping, data management, social sciences, economics, communication, business, local communities, politics etc. There are opportunities to involve our students working in collaborating teams with this conservation medicine approach.

The Wildlife Disease Association is an international multidisciplinary scientific organisation working in this area, please explore their website and consider joining.

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**A History of Infectious Diseases and Civilization  
: Conquest or Co-existence?**

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A burning question arose out of my studies and practice in infectious diseases over last two decades. That question is, “**Will humanity be victorious in its fight against infectious diseases?**” In other words, are we justified in our efforts to eliminate infections, or should we explore a path of coexistence? This presentation examines the complex relationships between infectious diseases and human civilization from a global and historical perspective, moving from hunter-gathers to early civilization and finally our modern world, to offer guidance on the future of nature and humanity. Many aspects of epidemics created by society are explored in analysing the connections between infectious diseases and human society. My conclusion is that containment in the control of epidemics may amount merely to preparation for a major tragedy. We need to learn that “to live symbiotically with microorganisms is the product of compromise, and not necessarily the most pleasant of situations.” In short, there is no future for humanity without symbiosis. The future of human society should be planned afresh with this understanding in mind, that there is no ideal balance, only an uncomfortable compromise with infectious agents and their consequences.

**Conservation Medicine from Ecology –A Case Study on Behavioural Ecology of Japanese Black Bears**

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Key words: behavioural ecology, Japanese black bear, one health, *Ursus thibetanus*, veterinary medicine

In Japan, black bears occur in 32 (or 33) of 47 prefectures and the range has been expanding with increases in secondly forested areas and de-population of rural areas (Fig.1: Japan Bear Network 2014). The rough country-wide population estimate was made by Ministry of Environment in 2010 (i.e., 13,000~30,000), but with very wide confidence intervals. Thus, this figure seemed to be an underestimation, and the population appears to be stable and/or increasing.

Hard mast failures have led to massive intrusions of bears into human residential areas, where they seek anthropogenic foods as a substitute. Human injuries and deaths sometimes occur at high rates in Honshu, around 100 people have been injured annually, including a few deaths, during the past 10 years. Such conflicts give rise to negative attitudes towards black bears. In an extreme case in 2006, over 4,000 bears were killed as pests. With this status, 21 prefectures have carried out the Specified Wildlife Conservation and Management Plan on bears according to the National Wildlife Protection and Hunting Law.

With this background, I have been studying behaviour ecology of Japanese black bears both in Okutama Mts. and Nikko-Ashio Mts. since 1991, with cooperation of universities and research institutes. Findings from this study, regarding bear ecology, provide valuable and practical information to ensure appropriate bear management planning in each area. However, there is still a lack of knowledge about their physiology (e.g., reproduction, hibernation, metabolic rate, nutrition condition, food digestibility), though this information is very important to better understand bear ecology and improve their future management. Recently, I have conducted some physiology studies which would not be possible without the cooperation of veterinarians.

In this presentation, I am going to summarize my past studies, and then would like to introduce my recent challenges on physiology studies.



Fig.1 Japanese black bear distribution area as of 2013 (Japan Bear Network 2014): gray tone and black tone indicate the distribution in 2003 and in 2013, respectively.

**WELFARE, MANAGEMENT and CONSERVATION**

**Experimental Translocation Project of Five Captive-born Amur Leopard Cats (*Prionailurus bengalensis euptilurus*) at Seoul Zoo, in Ansan Wetland Park Area.**

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**Introduction:** Amur leopard cat, *Prionailurus bengalensis euptilurus* (Elliott 1871) is distributed in eastern Siberia, Manchuria, Korean peninsula and on the Tsushima Island in Japan. Seoul Zoo has reproduced 31 Amur leopard cats since 2001 from more than 10 founder parents rescued or caught in wild. The definition of translocation is the human-mediated movement of living organisms of wild or captive origins from one area, with release in

another. It can be a strategy to restore ecosystem integrity, especially when top predators are involved. To the authors' knowledge it was first case to release into the wild as captive born leopard cats.

**Materials & Methods:** Five captive born Amur leopard cats (*Prionailurus bengalensis euptilurus*) were released into the Ansan wetland park, Sihwa reclaimed area on March 21st, 2014. They were two male cats weighing 4.95kg (ID No. 9901), 4.00kg (ID No. 8660) and three females weighing 4.05kg (ID No. 8615), 3.15kg (ID No. 6291), and 3.60kg (ID No. 5857) respectively. They were raised by their parents with offsprings and never fed on live animals for its food until release. So they were trained to hunt live animals such as mice, mud eels, and pigeons in its enclosure for two months before release. Two of them were born in 2012 and three were born in 2013. General health examination, vaccination, deworming, and putting radio telemetry device were done to all individuals by zoo vets prior to release to the wild. We monitored their position information received from the satellites to analyze the patterns of habitat usage for 4 months at least. We collected fecal samples to analyze foraging pattern after release.

**Results:** Two of them were found dead one month after they were released. Another one was also found dead two month after releasing. On necropsy one male died of uncertain cause and one female died of starvation because it moved away from the Shihwa wetland area. It must have been hard to forage to survive. One was found dried carcass so necropsy was useless. One male and one female live in the reclaimed wetland area actively. The diameters of their territory are 10.5km in male and 2.0km in female. Food source of one female was analyzed through the morphological assessment after dilating and filtering 21 fecal collections from the area. Rodents and birds were the main source of its food.

**Discussion:** Amur leopard cats are the only feline species among wild mammals in Korea. There were few studies on captive born Amur leopard cats' translocation or reintroduction into the wild in Korea. This report is the first experimental release and post-release monitoring in Amur leopard cats reproduced in the zoo. Further study is needed to improve monitoring technique and survival rates after releasing.

**Keywords:** Amur leopard cat, *Prionailurus bengalensis euptilurus*, release, translocation

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### The Follow-up Survey of the Released Raccoon Dog (*Nyctereutes procyonoides*) by the Wildlife Rescue Project

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**Introduction:** *Sarcoptes scabiei* is the external parasite which infects with various kinds of mammals and induces a serious itch and skin infection. Zoos in Yokohama are engaged in a wildlife rescue project. This project is conducted by the following process: if people find a diseased or injured wildlife at a road or catch the one by a trap, they bring it to the zoo. Kanazawa Zoological Gardens has rescued a lot of raccoon dogs (*Nyctereutes procyonoides*) which were mainly infected with *S. scabiei* and released them to the wild after a complete recovery. However, the released raccoon dog had not been followed up. Therefore, the raccoon dog was inserted a microchip for individual identification and released to the wild, and then examined whether these raccoon dogs were re-rescued or not.

**Materials and methods:** The survey was conducted for 4 years. 96 raccoon dogs were inserted microchips (Trovan ISO type, Surge-Miyawaki Co., Ltd., Tokyo) in their quadriceps femoris muscle and released to the wild. A place where the raccoon dog was released was in forest near the original rescued place. On the other hand, some raccoon dogs were released in the mountain (Mt. Ookusu, Yokosuka) far from the rescued place to examine in an activity range.

**Results:** The present study revealed that 6 of 96 raccoon dogs (6.25%) were re-rescued and 1 of 6 was re-rescued twice. 170.14 days on average was a period between the release and the re-rescue.

In 1st year, 22 raccoon dogs were released in Mt. Ookusu which was far from the original rescued place. In 2nd year, 2 raccoon dogs were re-rescued: one was at the original rescued place (about 5.8km away from Mt. Ookusu) and another was at a different place (about 4.6km away from Mt. Ookusu) far from the original rescued place.

In 2nd year, 34 raccoon dogs (32 inserted the microchip newly and 2 was re-rescued in 2nd year), were released. 28 were released at the original rescued places and 6 were at Mt. Ookusu far from the original places. In 3rd year, 3 were re-rescued at the released place and 1 was at the places about 8.3 km far from the released places, although these 4 were released at the original rescued places.

In 3rd year, 44 raccoon dogs (42 inserted the microchip newly and 2 re-rescued) were released at the original rescued places. In 4th year, only 1 was re-rescued at the place about 5.3km far from the released places.

**Discussion:** The present study revealed that the number of the re-rescue case was 7 of 100 release cases for 4 years. The active range of the raccoon dog was examined by a distance between the release and the re-rescued place. 4 of 7 cases showed that the raccoon dog moved 6.0km on average (maximum 8.3km). The previous study described that a home range of the raccoon dog was 0.1 to 6.0 km<sup>2</sup>, and some of the subadult one was reported to move 10 km<sup>1</sup>. Therefore, the present study showed that some raccoon dogs moved a wide area, but it was within the maximum one which the previously described.

The wildlife rescue project has always been confronted with a problem associated with the release area. Generally, the rescued animal has to be released to the original rescued place. However, the rescued place is not suitable for the release frequently because Yokohama is the second populated city in Japan and most rescued place is in a residential area. Therefore, some animals have to be released at a different place. The present study showed the following. If the raccoon dog was released at the mountain far from the original rescued places, 1 of 2 has returned to the original place. On the other hand, if the raccoon dog was released at the forest near the original rescued place, 3 of 5 were re-rescued at the original rescued places, but other 2 were far from the original rescued places. Although the number of the re-rescued raccoon dogs was limited, the present study revealed that some raccoon dogs were active and moved in a wide area.

The period between the release and the re-rescued was 170.14 days on average. This result meant that at least 6 of all released raccoon dog could survive in the wild after a long treatment period in the zoo. The raccoon dog did not lose ability to survive in the wild even if the one was kept to be fed in the zoo.

**Keywords:** Microchip, Raccoon dog, Release, Wildlife rescue

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### Species Identification for a Rescued Wolf with Mitochondrial DNA Control Region

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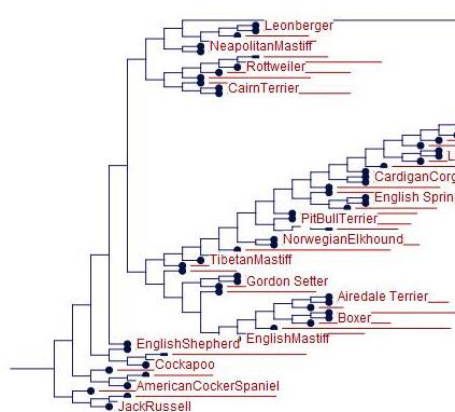
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**Introduction:** Wolf (*Canis lupus coreanus*) listed as class 1 endangered animals in Korea approved by the Ministry of Environment is thought to have been exterminated in the Korean peninsula. Not only in Korean peninsula, over the past decades, *Canis* species has been gradually endangered or extinct all over the world. Detecting the degree or extent of hybridization between species is important for evolutionary studies of speciation process, as well as for conservation biology studies.

We will review recent studies of species identification and distinction between wolves and dogs using mitochondrial DNA control region. Although distinguishing wolves from dogs using nuclear genes is difficult, the differences between dog and wolf can be evaluated by using canine mitochondrial DNA. For several times, it has been proved that wolf mitochondrial DNA control region haplotypes are not shared with any of the studied dog breed.

**Case:** “WOLF” is a suspected wolf who was rescued in 2012. He was found with radial and humeral fractures. Bandage management was kept for 147days until amputation proceeded. Since then, he is kept in Chungbuk wildlife rescue center.

**Materials & Methods:** Blood samples from 5 dogs, 1 wolf, 1 wolf-dog hybrid, and 1 suspected wolf were collected. All of the dog samples were collected from patients of Chungbuk national university animal hospital, while a wolf and a wolf-dog hybrid samples were provided by Cheongju zoo. As 1



suspected wolf, a permanent residence at Chungbuk wildlife rescue center was analyzed. DNA was extracted from the whole blood with EDTA using Invitrogen Dynabeads DNA DIRECT Universal kit. Control region was amplified by PCR using the primers designed by PALOMARES et al. (2002) (CR1 : 5'-CCACTATCAGCACCCAAAGC-3' and CR2R : 5'-CCCGGAGCGAGAGAAGAGG-3'). The reaction was carried out by TAKARA PCR thermal cycler using the following cycling parameters: The amplification program consisted of prenaturation (94°C 15min) followed by 31 cycles of denaturation (94°C 30sec), primer annealing (61°C 30sec) and extension (72°C 30sec) and final extension (72°C 3min). DNA fragment analysis was performed with BigDye@Terminator v3.1 cycle sequencing kits using Sanger method. The sequence data were analyzed with CLC Main Workbench 7.0.3. Phylogenetic trees were constructed by the

neighbour-joining (NJ) method.

**Results:** We analyzed approximately 330 base pair sequence of the mitochondrial DNA control region of 5 dogs, 1 wolf-dog hybrid, 1 wolf, and 1 suspected wolf. We successfully identified every individual's mtDNA control region haplotypes using CR1 and CR2R primers.

**Figure 1. Neighbor-joining tree, using complete genome of various dog breeds registered at GenBank, showing phylogenetic relationship among dogs, wolf-dog hybrids, and wolves.**

This phylogenetic tree contains haplotype sequences from the GenBank and 8 individuals' haplotype data from this research. The wolf (W1) from the zoo is clearly separated from other clusters, while gray wolf from other countries were grouped into one cluster. Wolf-dog hybrid (WK9) from the zoo and the suspected wolf (W2) locate at the tip of the wolf cluster.

**Discussion:** We analyzed approximately 330bp sequence of the mitochondrial DNA region of 8 individuals and found there are slight differences between the domestic dogs and wolves. Based on the analysis of the control region sequences, the suspected wolf has identified as a wolf or wolf-dog hybrid whose mother was a wolf. On the other hand, for the wolf clone, the domestic female dog was used as an oocyte donor and recipient, due to the limitations of obtaining gray wolf oocytes. This approach resulted in a hybrid embryo, containing nucleus DNA from donor cells and mtDNA from oocyte donors. Since the surrogate mother of a wolf clone was a dog, mitochondrial DNA of dog was inherited. From figure 1, wolf clone is clearly far from the other dog groups, but still obviously separated from wolves-involved clusters. It shows lineage of dogs and wolves can be distinguished by mitochondrial DNA. The lineage of a wolf-dog hybrids from the zoo is not clear, however, we assume that the individual was born between a female wolf and a male dog from the result of this research and from the reason below.

Hybridization between wolves and dogs has been observed in the wild according to several studies. Wolves continue to influence the genetic diversity of dogs. In consequence, wolf genes will gradually diffuse into the dog population at large. According to the research, most wolf-dog hybrids are a result of a female wolf mating with a male dog because of the physiological differences of mating season between these two species. While wolves have a well-defined mating season, dogs can often reproduce twice in the same year, and male dogs show high testosterone levels during the entire year. This makes male dogs able to fertilize all female wolves, while male wolves are unlikely to be sexually active at the time most female dogs are receptive. Since mitochondrial DNA is inherited from the mother, it will not be detected in case the paternal parent was a dog. Instead, paternal ancestry can be investigated using nuclear markers such as microsatellite loci. From this research, sequences provided by previous study can be used as a reference for species identification.

**Keywords:** dog, *Canis lupus familiaris*, wolf, *Canis lupus*, mitochondrial DNA, PCR, wolf-dog hybrids

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#### Reproductive Behavior of Caspian Red Deer (*Cervus elaphus maral*) in Wildlife Refuge of Semeskande, Sari

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**Introduction:** Caspian red deer or maral (*Cervus elaphus maral*) is the largest type of deer in Iran. Maral in the past has lived in the north forests of Iran from the Caspian Sea coast, Alborz Mountains Chain and oak forest of Zagros margin from the Azerbaijan up to Fars Province. But the generation of them was completely destroyed in the North West and West of Iran. According to reports about 50 years ago, they have been observed in Arasbaran forests and now a days populations of this species live in protected areas and out of reach of humans. Initially in 1973 Semeskande Wildlife Refuge with



an area of 1041 acres was placed under the protection then in Act No.63 in 1975 was introduced as wildlife refuge. This region is located in southeast of Sari and similar to plains forest lands is low altitude.

**Materials & Methods:** The present studies have been conducted and data were collected from 2006 to 2011 in the Wildlife Refuge Semeskande, Sari (one of the sites that preserve the maral in the Free Zones of Hyrcanian forest). Male (hart) and female (hind) maral deer reproductive behaviors were evaluated and recorded during reproductive season.

**Results: Male maral deer reproductive behaviors** Hart (stag or male deer) has 250 kg weight and with calculation of antler has 140 cm height. The antler fall in mid-March and then sprouts of antler are reactivated again in mid-spring. Growth of antlers continues in spring and is accompanied with soft cover of fluff. By increasing testosterone levels in blood of male maral deer, the antler cover becomes rigid and the hart instinctively rub his horn in order to sharpen the edges and mark his territory on the tree trunk. Urine splashing and genital secretions under the belly and even the neck are the events observed in this season that cause odor and dark color in those areas. **Female maral deer reproductive behaviors** There is a billion sperm in each milliliter of semen in natural mating of hart. The average amount of semen in male maral is 3.5 milliliter that the frequency and severity of mating affects these parameters. Usually if the first reproductive cycle of hind is along with ovulation, the mating leads to pregnancy. If pregnancy occurs, it's duration will be  $247 \pm 5$  days.

**Discussion: Male maral deer reproductive behaviors** This phase of antler growth is called velvet phase. Antlers in this phase are very sensitive and hyperemic, for this reason, males are placed at a distance from each other. From late June to mid-August antler's fluff is shedding and antler become calcareous. Hart start to rub their antlers on trees or hard objects. This structural change of antler in hart is due to fluctuations in testosterone. **Female maral deer reproductive behaviors** Coinciding with prolongation of darkness period (beginning of autumn) melatonin levels is increased in the blood of maral deer. This increase in melatonin leads to activation and increased sensitivity of gonadotropin receptor in pituitary gland and hypothalamus and this action is led to start of sexual activity and ovulation in female maral

**Keywords:** Caspian red deer, Reproduction, Behavior, Iran

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**Fig 2.** Maral deer male drag itself along the muddy pits in the forest during breeding season, this behavior is devoted to this season and called “damp spot wallow”



**Fig 3.** Predominance behavior of strong male maral over the weaker male in Hyrcanian forest



**Fig 4.** Mating behavior of maral deer in Wildlife Refuge of Semeskande, Sari



**Fig 5.** Parturition behavior of female maral deer in Wildlife Refuge of Semeskande, Sari

**Orangutan Veterinary Advisory Group (OVAG)**  
**An International Vet Network to Safe Orang-utans**  
 Drh. Fransiska Sulisty, MVS  
 OVAG steering committee, Malaysia

Key word: *veterinarians, orangutan, network.*

In 2009, the Orangutan Conservancy (OC) initiated a workshop to bring together orangutan vets and those interested in orangutan health issues. The delegates at this first workshop created the Orangutan Veterinary Advisory Group (OVAG).

The ethos of OVAG is improving orangutan health through collaboration. Many OVAG members working in over 10 rescue/rehabilitation centers and sanctuaries in Indonesia and Malaysia, altogether are taking care for more than 1000 animals. Other members are those working with orangutans in the wild, in zoos and other conservation organizations and in universities. Limited basic knowledge and remote working locations are among other of major obstacles faced by these vets

Since that first meeting in 2009, OVAG workshops have been conducted annually, and mainly serve as a continuing education program for vet, facilitate networking, as well as facilitate knowledge exchange among wildlife vets all around the world, focusing on orangutan health.

Topics that have been covered include: management of tuberculosis infection, management of Hepatitis B infection, disease risk analysis, field laboratory diagnostic, parasite control, nutrition, animal welfare and ethics, epidemiology, scientific writing, emergency medicine, and many more.

Practical training is also important. Sessions have been held are tranquilizer darting systems, emergency CPR, ultrasonography, post mortem procedure and orthopaedics. Clinical cases from various rescue/rehabilitation centres are also presented by vets to cover medical and management aspects and challenges of working in remote areas.

OVAG is now looking to collaborate with Indonesian and Malaysian universities to set up a post-graduate course program to acknowledge, certify future wildlife veterinarian / conservation scientists with a specific course for wildlife and ecosystem health management. These courses will in part utilize the vast amount of expert knowledge and teaching materials generated by OVAG members.

OVAG follows the training model set up for the Pan African Sanctuary Alliance (PASA) and together they form a unique, successful and evaluated training model for all interested in primate health

**Early Pregnancy Diagnosis in the White African Rhino using Ultrasound in Taman Safari Indonesia – Bogor**

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**Abstract**

Early pregnancy diagnosis is very important to enhance the successful of breeding program. Ultrasound scanning could provide direct image of the reproductive organs, therefore it could be used to assess the development of pregnancy immediately after mating. Early pregnancy diagnosis is very important to recognize the loss of conceptus as early as possible in order to determine the appearance of early reproductive disorder. Two white African rhinos were examined using ultrasound to monitor the pregnancy. The earliest pregnancy could be detected on the day-14, when the vesicle of conception appeared as black sphere was observed with diameter about 9 mm. Dorsal and ventral specular echo could be detected on the day-21. Embryo was seen first time on the day-28, while thickening of uterine wall was determined on the day-28. Fetus was developed progressively after day-35, and heart beat was recognized clearly on the day- 49 and 51. Size of fetus was measured about 13.7 mm at day-35, and it was developed up to 31 mm at day-56. Early pregnancy could be detected using ultrasound as early as conceptual vesicle was observed.

*Key words: Early pregnancy diagnosis, ultrasound, white African rhino*

**Diagnosis and Management of Gastric Foreign Body Obstruction Ingestion in Penguin in a Zoological Collection Lee Foo**

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**Abstract**

**Introduction:** This presentation is to share our experience in diagnosis and management of gastric foreign body ingestion in our colony of penguin. Clinical presentations, diagnostic work up, management and outcome of these cases are discussed.

**Materials & Methods:** Management of six cases of gastric foreign body ingestion involving Gentoo penguin (*Pygoscelis papua ellsworthi*) in our collection were reviewed.

**Results:** All these cases were diagnosed based on clinical presentation, physical examination, radiograph and gastroscopy examination. Endoscopic retrieval of foreign body resolved all the cases.

**Discussion:** Foreign body ingestion is a very common problem for penguin in zoological setting. Common presenting signs include depression, isolation from group, inappetance, regurgitation and vomiting. In our experience, lameness is an important clinical sign to alert attending veterinarian to a foreign body ingestion differential. Radiography and endoscopy are the imaging tools used for diagnosis and managing these cases, with satisfactory outcome.

**Key Words:** Penguin, foreign body



## Eclipse Plumage as one of the Characteristic in Identification of Red Junglefowls (*Gallus gallus*)

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### Abstract

An investigation was carried out to ascertain the existence of Red Jungle fowl (*Gallus gallus*) populations in oil palm plantations. This study took place in several oil palm plantations in Peninsular Malaysia and captured using the scoop netting method. Twenty seven Red Jungle fowls which comprised of seven adult males, six adult females, four juvenile males, three juvenile females, four male chicks and three female chicks were successfully captured including four male in their eclipse plumage throughout this study. The morphological study suggested the purity of the birds based on the characteristics exhibited. Four of the adult males were in their non breeding plumage or eclipse plumage. Further investigations revealed results which support the birds still possessed the pure morphological characteristics with one of main characteristic is eclipse plumage. The eclipse plumage or also known as the non breeding plumage which is generally found in adult male were observed to be have more black feathers unlike in the breeding season where the males are more colourful. The eclipse plumage is one of the characteristics that only occur in the pure Red Jungle fowl.

**Keywords:** Red Junglefowl, Morphology, Eclipse plumage

### Introduction

Red Junglefowl (*Gallus gallus*) is believed to be the origin of all present domestic chicken and it is indigenous to Southern and Southeast Asia. Generally, it can be identified through certain phenotypic characteristics such as the boat-shape body appearance, white or red ear lobe, and slender greyish blue leg. The sexes are highly dimorphic where the presence of seasonal eclipse plumage is more prominent in males compared to the females. The male assumes eclipse plumage, shedding the golden neck hackles and elongated tail feathers, blackish head and neck, the comb and wattles reduced in sized and paler in coloration (Madge, S. and McGowan P., 2002). The adult eclipse plumage male always been mistakable as juvenile, but the proven of maturity of the male is proof by appearance of the spur on the legs. The eclipse plumage occur one yearly for mature males within June to September (Madge, S. and McGowan P., 2002). This eclipse plumage is a morphology indicator of the pure wild Red Jungle fowl where it will not happen in crosses or hybrid traits (Hawkins, W.P., 1987). The eclipse plumage are occurred in the female too but due to non obvious breeding colour in females, the event is not been noticed. Therefore, in this study, the pure of Red Jungle Fowl can be determined by characteristic of the eclipse plumage especially in the male bird.

### Method / Experimental

This study was conducted in several oil palm plantations owned by government agencies, private companies and individual smallholders particularly in the state of Johor, Selangor, and Pahang, with hunting permit approved by the Department of Wildlife and National Park (PERHILITAN). Random sampling method was done whereby the birds were caught using scoop netting method. This study was performed from February to August in 2009 and 2010. The birds were handled properly to reduce stress and the morphological study was conducted through visualisation and the picture profiles and certain measurements were taken. Prior to the release, the spur was marked to prevent recapture. The morphological characteristics of village and decoy chicken were used as comparison.

### Results and Discussion

Collectively, twenty seven birds were successfully sampled from the three states. The birds comprised of seven adult males, six adult females, four juvenile males, three juvenile females, four male chicks and three female chicks and all the birds were captured by scoop netting method. Morphological identification showed that most of the birds depicted typical characteristics of Red Junglefowl as described in previous studies (Brisbin and Peterson, 2007; Salehatul, 2009; Madge and McGowan, 2002; Babied, 2009; Johnsgard, 1999). The birds possessed boat-shape body with horizontal posture, slender slaty blue tarsus and white ear lobes. Among the adult males captured, four of them demonstrated seasonal eclipse plumage. The eclipse plumage is occurred only once a year in adult male. The young males only reach the eclipse plumage after mature age estimated at more than one year. The results were expected as the previous studies reported from several authors (Johnsgard, 1999; Madge, and McGowan. 2002; Babjee 2009). The eclipse plumage or also known as the non breeding plumage which is generally found in adult male were observed to be have more black feathers unlike in the breeding season where the males are more colourful. It is always been mistaken as a juvenile male due to it colouration and size of their comb and wattles. The size of the comb and wattles were smaller when compared between the breeding adult male and the juvenile male. But, to confirm that it is an adult male, the spur should be seen clearly on both feet. The eclipse plumage is one of the characteristics that occur only in the pure Red Jungle fowl.

### Conclusion

In conclusion, this study is in accordance with previous reports on the presence of Red Junglefowl populations in the oil palm plantations (Abdullah and Babjee, 1982; Azhar et al., 2007; and Platt et al., 2009). One of the diverse characteristic is eclipse plumage which can be found on the Red Jungle Fowl. It is believed to be occurs in both male and female but more prominent to be seen in male. This special characteristic cannot be established in the cross breed or hybrid of red jungle fowl. Identification of one of characteristic of the pure Red Jungle Fowl can be judge by their eclipse plumage appearance as describe by Hawkins, W.P (1987).

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- SALEHATUL, K M A (2009). A major factor for the high species diversity in Indochina is the presence of limestone karsts, which cover an area of about 400,000 square kilometers, and are most extensive in Vietnam and Laos. Karst systems provide a variety of distinct microhabitats and are noted for their high levels of local endemism. Therefore they are recognized as ideal natural laboratories for ecological, evolutionary, taxonomic, and biogeographical

## Herpetological Exploration in Karst Forests of Laos

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### Abstract

The highest global diversity is found in tropical forests, which contain over 50% of Earth's known species and an even larger number of undescribed species. Tropical Asian forests evolved in isolation since the Tertiary and harbour a particular unique flora and fauna. Among these, the Indo-Chinese region stands out as one of the most significant biodiversity hotspots of the world with more than 7000 endemic plant species and over 80 endemic threatened vertebrate species. A major factor for the high species diversity in Indochina is the presence of limestone karsts, which cover an area of about 400,000 square kilometers, and are most extensive in Vietnam and Laos. Karst systems provide a variety of distinct microhabitats and are noted for their high levels of local endemism. Therefore they are recognized as ideal natural laboratories for ecological, evolutionary, taxonomic, and biogeographical

research. However, limestone karsts are also prone to degradation from quarrying for cement used in building and road construction, particularly in the Indochina countries.

In the Indochina region, the herpetofauna diversity of Laos is still poorly studied and at least 15 new species of reptiles and amphibians have been described from this country since 2010. At the same time that unprecedented numbers of new species are being discovered, however, reptiles and amphibians are threatened by extinction. Being predators at the upper end of trophic pyramids and due to their territoriality, and limited locomotion, reptiles and amphibians cannot evade disturbances, and active recolonization after local extinction is slow. On the other hand, we lack basic data (e.g., community composition, diversity, distribution) to inform conservation efforts. Our research focuses on the diversity of reptiles and amphibians in unexplored or poorly studied limestone forests in Laos. A wide range of elevations and the complexity of landforms have given this country a great diversity of natural habitats and a high level of biodiversity potential, in particular the karst inhabitants like geckonids and viper snakes.

## ZOO AND AQUARIUM VET NETWORKING

### Intestinal Mucinous Adenocarcinoma in a Clouded Leopard

(*Neofelis nebulosa*)

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**Keywords:** Intestinal, mucinous adenocarcinoma, clouded leopard

**Introduction:** Intestinal adenocarcinoma, is a malignant epithelial cell tumor, originates from enterocytes or goblet cells. The tumor spreads via intramural or submucosal lymphatic vessels. It is usually found in aging human and dogs. Adenocarcinoma in dogs and humans mainly locate in large intestine, but it locates in small intestine in cats. Common clinical signs are anorexia, diarrhea, weight loss and gastro-intestinal tract obstruction. MUC-2 antibody is usually used for confirmation and prognosis in this tumor in human cases, because mucin is associated to cell growth regulation, immune recognition and cellular adhesion, affecting to the invasive and metastasis abilities of cancer cells.

**Materials and methods:** A 14-year-old male clouded leopard had chronic diarrhea with no response to any treatments. Radiograph showed abdominal mass. Surgical exploration was performed to examine abdominal cavity. The numerous masses were found in many abdominal organs. The leopard was euthanized and submitted the postmortem examination. The attached, varied in shape and size masses, were collected for histopathological examination as well as heart, lung, liver, kidneys, small intestine, large intestine, and omentum. Periodic acid-Schiff-alcian blue (PAS-AB) staining was performed. Moreover, the sections were also stained for MUC-2 and gastrin immunohistochemistry.

**Results:** The body condition was emaciation with abdominal distension. The numerous masses were multifocal, various-sized with light yellow color in lung, spleen, omentum, intestinal wall and liver. There was focal invasive mass in intestinal lumen which caused narrowing lumen. Caseous material and catarrhal content were found in stomach and the intestines. Histopathologically, intestinal lumen was invaded by poorly circumscribed masses involved mucosa and muscular layers. Tumor cells were columnar and cuboid in granular structures and also showed atypia with various-sized nuclei and prominent nucleoli. Tumor cells contained and produced pink and pale-blue mucinous-like material. Tumor cells also invaded in hepatic and exocrine pancreatic parenchyma. Increasing of mesengial cells and infiltrating of mononuclear cells were observed in kidneys. In addition, mild telangiectasia and marked renal tubular degeneration were noted. Mucoid cysts formed in omentum and intestinal serosa. PAS-AB histochemical staining revealed that the contents in tumor cyst formation and cytoplasm of tumor cells were in both magenta and blue colors. Furthermore, the tumor sections were negative for human gastrin and MUC-2 immunohistochemistry.

**Discussion:** The PAS-AB staining showed that mucinous materials were composed of mixtures of acidic and neutral mucins because of positive magenta and blue staining. Mucin is glycoproteins which are synthesized by many epithelial tissues. Normally, the MUC-2 is used for mucin detection. However, sometimes human MUC-2 antibody was negative for mucin detection approximately 35%. In this case, MUC-2 was negative. This result might be caused by the non-specific antibody. The glandular structures, atypical cells, mucinous-like materials characteristics and immunohistochemical results were definitively diagnosed as intestinal mucinous adenocarcinoma. Normally, intestinal adenocarcinoma is extremely rare in exotic animals or wildlife. Intestinal adenocarcinoma cases in lion and clouded leopard were previously reported. The cancer derives from undifferentiated cryptal cells which they may normally differentiate into enterocytes or goblet cells. Primary tumor site cannot be concluded in this case because of massive metastasis. The metastasis and intestinal lumen stenosis are main reason to euthanasia for the quality of life. Ultrasonography and radiography should be performed in older animals with non-responsive gastrointestinal problems for early diagnosis.

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### Pathology and Molecular Characterization of Feline Parvovirus isolated from Small Indian Civets (*Viverricula indica*) in Thailand

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**Introduction:** Feline parvovirus is the carnivore subgroup of genus *Parvovirus*. Previous studies have been reported the parvovirus infection in various families of wild animals such as *Felidae*, *Canidae*, *Mustelidae*, *Procyonidae*, *Viveridae* and Order Primate. In September 2013, severe acute hemorrhagic diarrheic civets were observed at an opened system farm enclosing 52 small Indian civets (*Viverricula indica*), which is located in Trat province, Thailand. This Parvovirus-like symptom has been occurred in the beginning of rainy season. Initially, two of them died with bloody diarrhea, vomiting, associated dehydration and seizure. Consequently, the mortality rate of this outbreak was increased up to 67% within a month after the onset of disease. This study aimed to describe pathological and molecular characterization of parvovirus infection in the farmed civets in Thailand.

**Materials & Methods:** Twenty-five small Indian civets from a farm in Trat province, Thailand, were obtained in this study. Animal information including signalment, farm management and clinical history will be recorded. The tissues from 7-died civets with severe bloody diarrhea were collected in 10% neutral buffered formalin after routinely necropsy at Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University. Histological process was performed and stained with Hematoxylin and Eosin (H&E). Macroscopic and microscopic changes were systemically recorded. Other 18 fecal swabs from lived civets were preliminary tested by using canine parvovirus (CPV)/corona virus (CCV) and canine distemper virus (CDV) rapid test kit (Bionote®, Korea). For the genetic detection and sequencing analysis, conventional PCR were used with a set of primers comprising of eleven primers, which modified from Mochizuki and colleagues (1996). Complete VP2 gene of feline parvovirus and deduced amino acids were analyzed by MEGA6 program.



**Result:** The farm management handled in opened system and had poor biosecurity. Individual ventilated cages were made by wood; thus all animals were able to directly expose to environment, people and other animals. After the outbreak has emerged by the occurrence of two civets died with bloody diarrhea, the other subclinical ill with depression and low appetite were then separated into the distinct area. Submitted eighteen live civets included mild depressive sign (n=7), stagger while walking and bloody diarrhea sign (n=1), and the other 10 civets showed no symptoms. All of them were negative against CDV and CPV/CCV test kits except one civet was positive to CPV (5.56%). Histopathologically, 7 died civets demonstrated pathognomonic lesions of parvoviral enteritis throughout the intestine including villous atrophy, fusion and desquamation of villous epithelium, cryptal necrosis. Lymphoid depletion in Peyer's patches and mesenteric lymph nodes were observed. Viral amphophilic intranuclear inclusion bodies were found in cryptal epithelial cells, enterocytes, esophageal epithelial mucosa, hepatocytes and bronchial epithelial cells. Thirteen fecal specimens showed a specific fragment of VP2 gene by using PCR (72.2%, 13/18). Genetic characterization and deduced amino acids showed identity of 99% with reference feline panleukopenia virus (FPLV) deposited in GenBank.

**Discussion:** This is the first report of feline panleukopenia virus outbreak in farmed civets in Thailand. Also known that FPLV cannot infect and cause disease in canids, but it restrict to domestic and captive felids. Moreover, Parvovirus-like symptoms have shown in various carnivore species, which are infected with FPLV in the close related families such as *Mustelidae*, *Procyonidae* and *Viveridae*. This outbreak in civet farm indicated that trans-species infection has occurred increasingly between *Felidae* primary host and other species especially with *Viveridae* during a half of decade ago. The transferrin receptor (TfR) is an important role of host selection to infection of this virus. Whether TfR characterization of *Viveridae* could play the same role of the FPLV infection as similar in *Felidae*, it needs to be clarifying in the future. In addition, the biosecurity of farm should be concerned. The opened farm setting is the critical point that other animals can pass throughout the farm all the time as well as the personal clothes and movable goods using in the farm should be aware. The healthy cat also can shed its FPLV pathogen via feces for several months.

**Keywords:** Feline panleukopenia virus, Parvoviral enteritis, Pathology, Small Indian civet, VP2 gene, Thailand

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#### Reptilian Paramyxovirus in Captive Boidae and Pythonidae

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**Introduction:** Reptilian paramyxovirus (rPMV) is one of most important viral infection that occurred worldwide in reptiles especially in Boidae and Pythonidae snakes. The members of rPMV are Sunshine virus and Ferlavirus. The infected snakes may show clinical signs related with neuro-respiratory disorder. There were many suspected clinical cases that could not specify the causes. So far, there is no information and available diagnostic laboratory to identify these viruses in Thailand.

**Materials & Methods:** Thirty-five snake carcasses with history of neuro-respiratory problems were submitted to Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Thailand. Complete post-mortem examination was done. The tissue samples were fixed in 10% neutral buffered formalin, and routinely processed and embedded in paraffin blocks. The sections were cut and stained with hematoxylin and eosin (H&E) and special staining. PCR with two primer sets were performed to detect viral nucleic acid of Ferlavirus and Sunshine virus that might be causative pathogen. PCR products were submitted for genetic sequencing and phylogenetic analysis.

**Results:** All carcasses showed severe acute diffuse pneumonia with or without secondary bacterial infection. There were no remarkable lesions in the other visceral organs except generalized congestion. Histopathologically, most distinguished lesion of the suspected cases was numerous varied sizes of amphophilic to eosinophilic intracytoplasmic inclusion bodies (ICIB) in pancreas, liver, kidney, spleen and brain. All selected sections showed strongly positive to Methyl green-Pyronin Y special staining that used for viral RNA identification. On molecular study, nine of 21 suspected cases showed specific amplicons that similar to the positive Sunshine virus control. One of nine positive products showed a homology sequence to Sunshine virus following blast to the published sequences in GenBank.

**Discussion:** This study is the first report of rPMV infection in Boidae and Pythonidae snakes in Thailand. Sunshine virus was successfully identified by using RT-PCR and genetic sequencing. No remarkable lesion was noted from gross pathology while viral inclusions were marked in multiple organs obtained by histopathology. Molecular study showed that one of nine PCR positive samples displayed sequence homology with Sunshine virus while the other eight sequences shared homology in group but not to Sunshine virus. This may refer to new strain of rPMV. However, advance technology such as next-generation sequencing may obligatory for further study.

**Conclusion:** Histopathology and molecular techniques obtained by this study are appropriate diagnostic tools for rPMV identification. The diagnostic laboratory is established for rPMV detection and differentiation between Sunshine virus and Ferlavirus. In addition, molecular technique might be used as a screening test in clinical cases that showed suspected clinical signs on neuro-respiratory system. This could help veterinarians and owners for disease prevention and epidemiologic control.

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**Keywords:** Inclusion body, Pathology, PCR, Snakes, Sunshine virus

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#### Metabolic Bone Disease in a Veiled Chameleon (*Chamaeleo calyptatus*)

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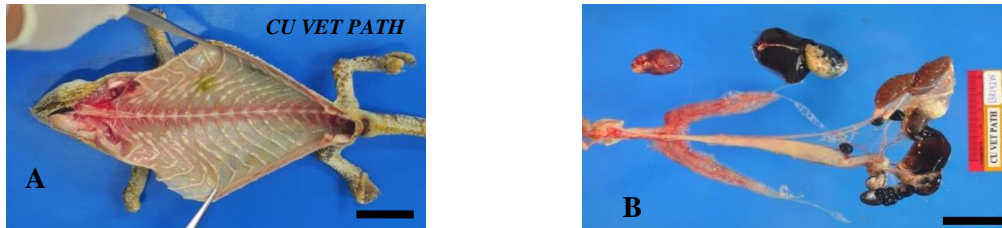
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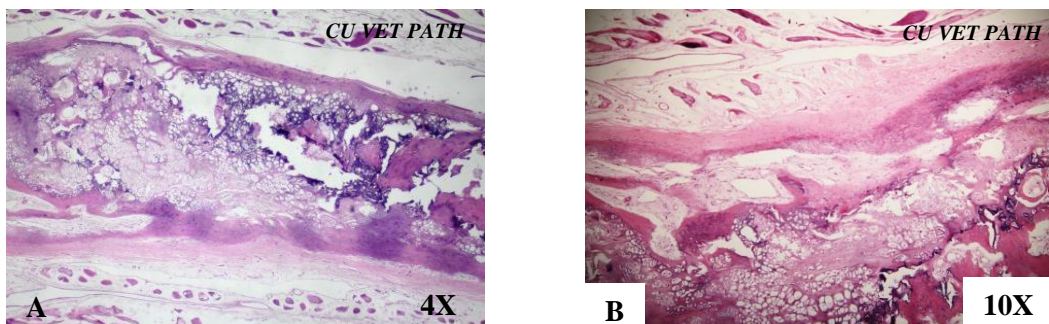
**Introduction:** Metabolic bone disease is frequently seen in captive reptiles especially in lizards. The mechanisms of disease are associated with low dietary levels or vitamin D, an improper ratio of dietary calcium and phosphorus, or inadequate exposure to ultraviolet light followed by the effects of secondary hyperparathyroidism. There are many factors involved in this condition, but inappropriate husbandry is the most important factor involved in this condition.

**Materials & Methods:** A 5-year-old male Veiled Chameleon (*Chamaeleo calyptatus*) was only fed with fruits and vegetables in the first two years of age and lived indoor with 12 hours of sun basking. The animal was severely dehydrated and emaciated. Routine necropsy was performed at the Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University. Briefly for histopathological slide preparation, the tissue samples were fixed in 10% formalin and embedded in paraffin. The paraffin blocks were sectioned at 4 micron thickness and stained with hematoxylin and eosin (H&E) and special stainings; Masson's trichrome, Periodic acid Schiff (PAS) reaction and Von Kossa.

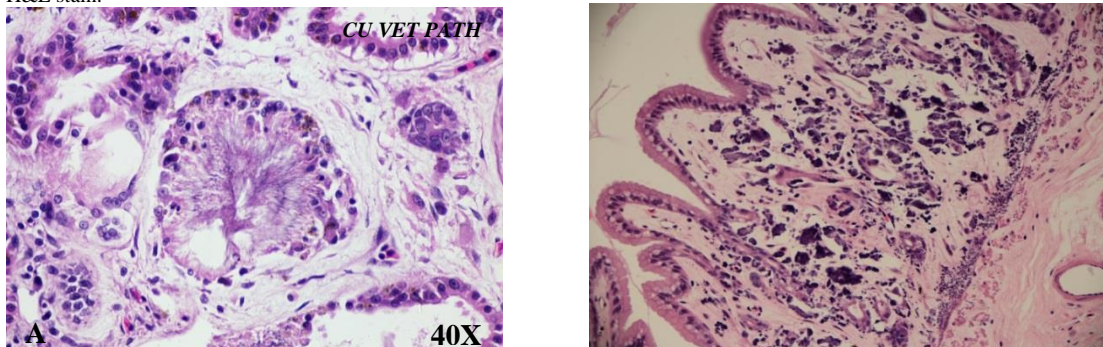
**Results:** Macroscopically, there were multifocal to coalescing proliferative osteopathy of the ribs (Fig.1A), granulomatous nephritis and cholecystitis with white crystal deposition (visceral gout) (Fig.1B). The histopathological diagnoses were generalized fibrous osteodystrophy (Fig.2), chronic multifocal gout granulomatous nephritis (Fig.3A), severe multifocal glomerulosclerosis demonstrated by PAS and severe diffuse metastatic calcification of the stomach and intestines (Fig.3B). The special staining; Masson's Trichrome and Von Kossa, demonstrated fibrous tissue replaced bone and calcium deposition in renal tubule and at the lamina propria and submucosa of the stomach and intestines consequently. Lungs had no evidence of metastatic calcification and had no other remarkable lesions.



**Fig.1** (A) Multifocal to coalescing bosselated osteopathy of ribs (B) The white crystalline deposit on serosal surface throughout the gall bladder and kidneys (bar = 3 cm)



**Fig.2:** Histopathology of rib; (A) the remodeling space formerly occupied by calcified bone, filled by fibrous connective tissue with marked disappearance of osseous tissue. (B) There were partial to complete replacement of normal compact and cancellous bone by numerous loosely arranged fibroblasts. H&E stain.



**Fig3.** (A) Kidney: gout granuloma (fan shape tophi) surrounded by inflammatory cells (arrow). (B) Intestine: Diffuse deeply basophilic calcium crystals deposited at the lamina propria and submucosa.

**Discussion:** The cause of metabolic bone disease in this animal originated not only because of lacking of calcium and vitamin D supplementation in young growing stage, resulting in bone resorption and release of calcium and phosphate from bone, but also reduction in functional nephrons to increase bone resorption, then followed by the replacement of fibrous connective tissue in the cancellous bones and metastatic calcification at the stomach and intestines (uremic gastroenteropathy). However, there was no lung involvement of the metastatic calcification that differs from the mammals' predominantly pathological sites.

**Keywords:** Veiled Chameleon, Metabolic bone disease

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### Causes of Deaths in Scimitar-horned Oryx (*Oryx dammah*) in the UK. Zoological Collections

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#### Abstract

This retrospective study was done to determine the causes of deaths and diseases affecting scimitar-horned oryx (*Oryx dammah*) in captivity. Necropsy reports of 420 scimitar-horned oryx died or euthanized due to medical reasons in 9 zoological collections between 1971-2012 in the UK were analyzed. Causes of deaths were determined for three age groups and the prevalence of recorded diseases were compared between the age groups and the gender. The main cause of neonatal deaths (32.1%, n=35/109) were stillbirths and premature births (perinatal). Bacterial infections were the most common cause for juvenile (36.8%, n=50/136) and adult (32.0%, n=56/175) deaths.

#### Introduction

The captive breeding programme of scimitar-horned oryx started globally in the 1960s and the majority of captive individuals are descended from animals wild-caught in Chad in the mid-1960s. Aalborg zoo, Denmark provided the British herd of scimitar-horned oryx, which arrived at Marwell zoo in 1971 (GILL and CAVE, 2008). It has been estimated that there are at least 1,550 captive individuals in these breeding programmes around the world. Currently, there are populations of scimitar-horned oryx in fenced protected areas in Tunisia, Morocco and Senegal (GILBERT, 2008). However, it is believed that only few scimitar-horned oryx are left in UK captive facilities.

It is assumed that this species is susceptible to most infectious and non-infectious diseases which affect domestic cattle, sheep and goats (FLACH, 2004). It is clear that since the scimitar-horned oryx is totally extinct from their historical range, survival of this species is totally dependent on the health and welfare of the captive populations.

#### Materials and Methods

Necropsy reports of scimitar-horned oryx, that died between 1971 and 2012 were requested from British and Irish Association of Zoos and Aquariums (BIAZA) zoological collections that currently hold or previously held this species. Zoological collections were approached with the support from BIAZA and scimitar-horned oryx European Endangered species Programme (EEP) coordinator and international studbook keeper. International studbook post-mortem data were also approached in an attempt to complete any missing data.

The major cause of death or euthanasia for each animal was categorized in to one of the nine categories including perinatal, infectious, trauma, neoplastic, nutritional, environmental, degenerative and unknown (Table 1). Scimitar-horned oryx were categorized in to three age classes and the causes of deaths were considered for each age categories; neonates (1-10 days), juvenile (11 days-11 months), adults (> 1 years). Prevalence of major diseases or conditions within each age group and within body systems was also determined.

#### Results

In the neonatal age category (0-10 days), a definitive cause of death has not been determined for 38.5% (n=42) cases. Majority of deaths (32.1%, n=35) were stillbirths and premature births for which the causes of deaths were not known except for five cases. Infectious diseases accounted for 9.2% (n=10) of neonatal deaths. Predominantly recorded infectious diseases include bacterial enteritis and generalized infections. Traumatic injuries, exposure to extreme weather conditions and nutritional related causes accounted for 6.4% (n=7). Reported causes for nutritional deficiencies were maternal neglect (n=3) and failure to suck (n=4) which resulted in starvation. One death was due to aspiration pneumonia following hand rearing.

In the Juvenile (11 days- 11 months) age group, 136 necropsy reports were received and definitive causes of deaths have not been determined for 69 (50.7%) cases. In the adult age group (> 1 year) full necropsy reports were received for 175 individuals and definitive diagnosis have not been made for 66 (37.7%) cases. Infectious diseases were the most common causes for death in both juvenile (36.8%, n=50) and adult (32.0%, n=56) age groups. The second most common cause for deaths of juvenile and adult age groups was trauma accounting for 10.3% (n=14) and 17.7% (n=31) deaths respectively. Other causes such as post anaesthetic complications, capture myopathy and neuropathy have accounted for third most common (6.9%, n=12) cause of death in adult scimitar-horned oryx while nutrition related causes are the third most common (1.5%, n=2) reason for juvenile deaths. However, nutritional related problems have accounted for 2.9% (n=5) of adult mortality while degenerative diseases and neoplasia each have resulted in 1.1% (n=2) deaths.

The most commonly encountered infectious were bacterial infections including gastroenteritis or enteritis, pneumonia, arthritis, peritonitis, hepatitis, osteomyelitis, meningitis, glomerulonephritis, hepatitis, myocarditis, cellulitis and myositis. *Escherichia coli* (E.coli) was the most predominantly isolated bacteria (5.7%, n= 24) from the cultured samples from animals with infectious conditions. *Streptococcus* species (1.7%, n=7.0), *Yersinia pseudotuberculosis* (1.4%, n= 6) and *Clostridial* species (1.7%, n=7) have also been recorded. In addition, yersiniosis has been suspected in two other cases with particular gross pathological lesions (small granuloma over the liver, spleen, intestine and necrosis of the intestine.)

#### Discussion

This is the first comprehensive study to document the causes of death, and disease and conditions present at the time of the death of scimitar-horned oryx in UK captive collections. The most significant pathological findings and the pathologist's comments on the findings were considered when determining the major cause of death. However, there could be variations in the examination techniques, diagnostic capabilities, and record keeping systems in individual institutions. Despite these limitations, the necropsy report analysed were important for identifying the common causes of mortality and some of the diseases affecting scimitar-horned oryx in captivity.

Overall, the most prevalent causes of deaths found in this study were infectious diseases and traumatic lesions in juvenile and adult age groups, whereas deaths due to stillbirths and premature births were the most common in neonates. In general, high prevalence of health problems in captive population of scimitar-horned oryx can affect the welfare in captivity and reintroduction programs of this species. However, necropsy reports would not provide details about all the diseases affecting this species since some of the diseases would not cause death of the animals or would not present at the time of death.

#### Conclusion

There were considerable numbers of cases for which the definitive cause of death was not been determined. Thus, standardizing the necropsy protocol and collections of more tissue samples at necropsy would improve the diagnosis of definitive causes of deaths. Furthermore, evaluation of dietary requirements of vitamins and mineral would provide guidelines to improve feeding management in captivity. Measures can be taken to prevent infectious diseases through improving the husbandry practices, strategic deworming, and supplementary feeding and prevent feed contamination during winter. When consider the overall prevalence of infectious diseases, perinatal deaths and the number of cases without a known causes of death, need for an evaluation of genetic management program of scimitar-horned oryx can be highlighted.

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## Pathological and Immunohistochemical Findings of Avian Encephalomyelitis Virus Infection in Captive Emu, Ostrich and American Flamingo

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## Abstract

**Introduction:** Avian encephalomyelitis, also known as epidemic tremor, is an infectious viral disease of birds naturally affecting young birds including chicks, pheasant, turkey, quail and pigeons. The etiological agent avian encephalomyelitis virus is a hepatovirus in family picornaviridae. Avian encephalomyelitis has not been recorded in Emu, ostrich and flamingo.

**Materials & Methods:** An eight-day-old Emu chick exhibited neurological signs such as ataxia, paresis and rapid tremors of the head and neck then died. One week later, 3 ostrich and one flamingo embryonated eggs failed to hatch were presented. One young flamingo dead with bumble foot and debilitation was also presented. The birds were dissected and processed for paraffin embedding and staining. Immunohistochemistry, polyclonal rabbit anti-AEV antibody was used to detect virus antigen in brain tissues using Two-Step Polymer Method (Dako EnVision™).

**Results:** The histopathological lesions were variable in intensity. It was weaker in embryonated eggs than chicks. Non-purulent meningoencephalitis was observed in Emu chicks with perivascular lymphocytic infiltration, gliosis, neuronal degeneration and necrosis. Neuronal lesions composed mainly of central chromatolysis and occasionally neuronal shrinkage as well as neuronal lysis. Motor neurons of medulla oblongata and neurons of nuclear groups of midbrain were mostly affected. Focal and diffuse gliosis was also observed in most of brain tissues. Similar lesions with milder degree were observed in brain from embryos of ostrich and flamingo. Immunohistochemically, AEV antigen was recognized in cerebral hemispheres, midbrain, cerebellum and medulla oblongata. Large and small neurons were stained positive besides glia cells. Granular and diffuse labeling in cytoplasm of neurons was observed.

**In conclusion,** these might be first record of AEV infection in emu, ostrich and flamingo. Moreover, we optimize the method of immunohistochemical staining of AEV as it has not previously used.

**Key words:** Avian encephalomyelitis virus, captive, Emu, Ostrich, Flamingo, Central chromatolysis, Immunohistochemistry.

## ASIAN ELEPHANT

### The Effect of Platelet Concentrate Gel on Wound Healing in Asian Elephant (*Elephas maximus*)

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**Abstract:** Platelet concentrate (PC) contains numerous growth factors that play a critical role in mediating healing. To study the effect of PC gel on wound healing in Asian elephant, twelve skin wounds were randomized into two groups. Treatment group were treated with PC gel and control group were treated with platelet-poor plasma (PPP) gel. Healing time, healing rate and wound healing score were not significantly higher in control group than those of treatment group ( $p > 0.05$ ). PC gel had potency to improve quality of wound healing in Asian elephant compared to PPP gel. However, more sample number and further investigation are required.

**Keywords:** Platelet concentrate gel Wound Elephant

**Introduction:** Skin wound is frequently found disorder in elephants. Their thick and dry skin cause delayed wound healing (Sukklad, 2006). Platelet concentrate gel is prepared from plasma. After platelet activation, growth factors and proteins are released from granules such as transforming growth factor- $\beta$  (TGF- $\beta$ ). These substances induce cell division, limit inflammatory process, increase vascularization, accelerate epithelial cell regeneration and boost the immune system (Lacci, 2010). No research has studied about platelet concentrate in elephant. The aim of this research is to study the effect of autologous platelet concentrate gel on wound healing in Asian elephant.

**Materials & Methods:** Wound samples were from healthy captive elephants at the age of 15 to 50 years old. The wound located at less movement area, not expose to muscle or bone and not more than 50 cm<sup>3</sup> of volume. Twelve elephant skin wounds were randomized into two groups. Treatment group (6 wounds) were treated with PC gel and control group (6 wounds) were treated with PPP gel. The elephants were kept in clean place. Wound dressing was performed daily by using nitrofurazone ointment and anti-fly powder after cleaning.

Gel application was performed every two weeks until complete healing. Gel was produced from elephant plasma. Blood was collected from marginal ear vein into a tube containing acid citrate dextrose (ACD) as an anticoagulant at the ratio of 6 to 1. The blood tube was kept in room temperature and transferred to centrifuge for platelet separation. After wound dressing, bovine thrombin was mixed into the PC or PPP to set a gel at the ratio of 10 to 1. The gel was applied on the wound immediately and covered with gauze for at least 1 hour.

Platelet functions were tested before gel production that is platelet count by automated blood cell analyzer, platelet aggregation test by aggregometer and TGF- $\beta$  quantitation by enzyme-linked immunosorbent assay. Wound healing was assessed weekly for wound size and wound healing score. By using vernier caliper, the length, width and depth were measured. To estimate the healing rate, the wound modified area (MA) was calculated from the quotient initial area (cm<sup>2</sup>) / area at the end of every week (cm<sup>2</sup>). For wound scoring, granulation tissue and discharge were included as the criteria (Table 1).

Statistical analysis was performed with the R project for statistical computing (R version 2.15.2). The Student's t-test was used to compare the healing time between groups. The Permutation test was used to compare the healing rate and wound healing score between groups. Values of  $p < 0.05$  were considered statistically significant.

**Table 1.** Wound scoring

Characteristics	Score			
	0	1	2	3
Granulation	Heavy	Moderate	Mild	None
Discharge	None	Serosanguineous	Purulent	

Adapted from Sylvestre, 2002



**Results:** Platelet count in PC ( $888.44 \times 10^3/\mu\text{L}$ ) contained around 3.78 times more than in whole blood ( $235 \times 10^3/\mu\text{L}$ ). The median percentage of platelet aggregation was 62 percent. The median TGF- $\beta$  concentration was 906.75 pg/ml. In PC group, the mean time to complete healing was shorter than that in PPP group but the difference was not significant (Table 2). The rate of wound healing rate was no significant difference between the two groups during two weeks period (Table 3). PC group had better wound healing score compare to PPP group at the end of the first, second and third week but the differences were not significant (Table 4).

**Table 2.** The mean time to complete healing

	PC group	PPP group	<i>p</i>
Mean (days $\pm$ S.D.)	21.17 $\pm$ 12.42	22.67 $\pm$ 12.37	0.838

**Table 3.** The modified area at the end of the first (MA<sub>1</sub>) and the second (MA<sub>2</sub>) week

MA	PC group	PPP group	<i>p</i>
Mean MA <sub>1</sub> (cm <sup>2</sup> $\pm$ S.D.)	2.53 $\pm$ 1.98	1.60 $\pm$ 0.23	0.409
Mean MA <sub>2</sub> (cm <sup>2</sup> $\pm$ S.D.)	18.89 $\pm$ 22.26	31.10 $\pm$ 45.78	0.699

**Table 4.** Wound healing score at the weekly time-points

Time-point	PC group	PPP group	<i>p</i>
Day 0 (mean $\pm$ S.D.)	3.33 $\pm$ 1.03	3.83 $\pm$ 0.75	0.530
Day 7 (mean $\pm$ S.D.)	1.83 $\pm$ 1.17	2.5 $\pm$ 1.38	0.516
Day 14 (mean $\pm$ S.D.)	1.17 $\pm$ 1.33	1.33 $\pm$ 1.21	1
Day 21 (mean $\pm$ S.D.)	0.33 $\pm$ 0.82	0.67 $\pm$ 1.03	1

**Discussion and conclusion:** The proposed protocol is adequate to achieve a PC gel with good features. No clinical complications were observed after PC treatment or during the healing process. This is the first study in aggregation test and growth factor concentration of platelet in Asian elephant.

Healing time, healing rate and wound healing score were not significantly higher in control group than those of treatment group ( $p > 0.05$ ). Several possible confounding variables exist in this study, including the variation in patient characteristics; patient age, wound etiology, initial wound area, wound depth, wound location, wound contamination and growth factor concentration. This study was also limited by the small sample size and the treatment period could be too short to observe substantial benefit. A dosage of platelet concentrate shown to induce optimal biological benefit is a 2-3 fold increase compared with peripheral blood (Anitua, 2009). In this study, PC contained around 3.78 times more platelet yield than whole blood. Hence, the ideal PC should present a platelet count of over 1,000,000 platelets/ml (Andrade, 2008). We used PPP gel as the control group while many authors used saline gauze, saline gel or no topical treatment. PPP gel, also known as fibrin glue or fibrin sealant, contains all of the clotting proteins; it forms a fibrin matrix when activated with an agonist. It has been shown that the fibrin matrix alone may enhance healing by providing a conductive scaffold for cell migration and new matrix formation (Amy, 2012). Further study may be needed to compare wound healing between PC, PPP and no topical treatment.

PC is prepared from the elephant's own blood; the risk of complications is minimal. Preparation of PC is quick, simple, and relatively inexpensive. This study may be the guidance for the further study of PC treatment on wound healing in elephants and other animals. Within the limits of the present study, PC gel had potency to improve quality of wound healing in Asian elephant compared to PPP gel. However, more sample number and further investigation are required.

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#### Immune Responses on Different Dosages of Tetanus Toxoid in Captive Asian Elephant (*Elephas maximus*); Preliminary Study

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**Introduction:** Tetanus is occasional fatal disease in animals and human, that caused by anaerobic gram positive rod bacteria named *Clostridium tetani*. The bacteria replicates and produces exotoxin in anaerobic condition, especially a contaminated or penetrated wounds. Tetanus toxin, tetanolysin, believed to conduct the pathogenesis of tetanus, and a major toxin as tetanospasmin which inhibited the neurotransmission by blocking a releases of neurotransmitter, glycine and gamma-aminobutyric acid (GABA). The mechanisms caused motor reflex inhibition of motor neurons, then a classical signs and symptom of tetanus could be observed as muscle spasms, lock jaw, cardiac arrhythmia, tachycardia, hypertension and respiratory failure. Tetanus diagnostic is quite limited, usually evaluated from history taking, symptom and serum creatinine kinase (CK). When an animal has suspected tetanus infection, they are treated with giving the passive immune as tetanus antitoxin to neutralize toxin, tetanus toxoid for immune system stimulation to generate antitoxin, antibiotic to eradicate an agent, and supportive treatments (Phuangkum *et al.*, 2005). The successful rate of treatment depends on any factors especially earlier tentative or final diagnosis and antitoxin response. Moreover anaphylactic shock could be found after tetanus antitoxin administration. Therefore tetanus prevention with toxoid vaccination would be an alternative method in several animal species that are susceptible to tetanus especially horses. The tetanus prevention program has been applied to captive elephants with products for horses or livestock (Fowler and Mikota, 2006). Although a previous scientific literature has reported that tetanus antitoxin titer can detected in vaccinated elephants whereof single dose, booster dose and different dose test, as well as transplacental antibody transfer. However the results were varied due to the elephant numbers, especially the different dosage evaluation (Lindsay *et al.*, 2010, Natalia *et al.*, 2011, Nofs *et al.*, 2013). Therefore the appropriate dose should be investigated with more elephant numbers for more information about tetanus antitoxin titer in elephants. Their suggestion was to recommend and/or establish the annual tetanus toxoid vaccination in routine husbandry for captive elephants. Beside that tetanus toxoid products are different in commercial brands, would be well suited for the elephant tetanus prevention in Thailand, if the available local toxoid product could be affected to stimulate elephant immune response. Therefore the aim of this study is to evaluate the immune response to various doses of commercial tetanus toxoid that available in Thailand, for suitable tetanus vaccination program in captive Thai elephant.

**Materials & Methods:** Twenty-seven healthy Asian elephants (*E. maximus*) at the age of 3-61 years old (20 females and 7 males), and historically free from tetanus toxoid vaccination, were used in this study. The elephants were divided to 3 groups with equal age and sex, and given 3 different dosages of commercial equine tetanus toxoid (Fort Dodge®, Zoetis, Thailand), 1 ml-dose group (n=9, age 27.2 $\pm$ 5.9), 2 ml-dose group (n=9, age 25.6 $\pm$ 5.3), and 3

ml-dose group (n=9, age 32.8±4.8). Toxoid vaccination program was performed as follow by recommendation of that commercial product. The blood samples were collected for pre-vaccinated samples from ear vein with an aseptic procedure on first day (day 0), before administrated an initial dose in each group. After that all elephant had follow by booster in same dose of initial vaccination one month later (day 30). Post-vaccinated blood samples were collected monthly interval after a first immunization on day 30, 60, 90 and 120, for analysis of tetanus antitoxin titer. Polyclonal rabbit antiserum to elephant immunoglobulin with horseradish peroxidase labelled was used to detect tetanus antitoxin in elephant sera by indirect ELISA in this study. All serum antibody results of ELISA were calculated to Sample to Positive ratio (SP ratio), and then compared mean between three dosage groups. According to the base line of elephants in this study were varied, therefore SP ratio were transformed to percentage for statistical analysis.

**Results:** The elephant immunoglobulin that against to tetanus toxin was detected after initial vaccination among 3 groups on day 30 with following a peak on day 60. However the antibody concentration was varied among individuals, and some elephants showed slightly response to tetanus toxoid immunization. The percentage SP ratio of highest dose group, 3 ml-dose tetanus toxoid, was higher than another dosage groups, but not significantly different between all groups ( $p>0.05$ ). The individual response showed that older elephants had strongly immune response than younger ones ( $p\leq 0.05$ ).

**Discussion:** The commercial tetanus toxoid for horse and livestock could stimulate the elephant immune response to tetanus toxoid, with a recommended procedure in horse toxoid vaccination program. Although the statistical results showed a response of 3 dosage groups was not significantly different. However the 3 ml-dose group seemed to be different to a 1 ml-dose group ( $p=0.062$ ), whereas it was equal to 2 ml-dose group in a responses. That trend implied the high dose of toxoid could be high efficiency than the low dose; however, further investigation for appropriated dose should be performed. Moreover when we focused on age, the older elephants (more than 41 years old) were figured out a greater response to toxoid immunization than younger ones. That was similar to previous report of Lindsay *et al.* (2010), the immunosenescence evidence was not observed in older elephants, the immune component of old elephant was fully functions in their physiology. The high immune responses in older elephants might be better primed from natural exposed to tetanus toxin prior vaccination. The reason could be explained by the results of excluded elephant subjects, they were suspected to tetanus and/or toxoid administrated in a years before start this toxoid vaccination program. Their immune responses were very high in a level and peak on day 30 and day 60 respectively which compared to a study groups. Beside that the natural exposed during period of study could be made the natural neutralization in some elephant that had low immune response. Another reason as internal and external factors (e.g. body condition, individual physiological status, stress) might be affected to low immune response elephants. Natalia *et al.* (2011) reported that the low immune response elephant was a captured elephant which poor body condition, and in training procedures. This study could suggest that the commercial horse tetanus toxoid vaccine can be used for prevent tetanus in captive elephant. However the appropriate dose and proper program should be more investigated by more elephant numbers especially varies in age, health and physiological status, as well as the effective antibody titer for protection tetanus in elephant.

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**Keywords:** tetanus toxoid, dosage, immunization, Asian elephant (*Elephas maximus*)

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#### Case Report: Cecal Trocarization for Gas Decompressing in Asian elephant (*Elephas maximus*)

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**Introduction:** Gastrointestinal (GI) problem is the major health problem of Thai elephant (*Elephas maximus*), which was reported to be 24% of cases in the National Elephant Institute's hospital during 2005-2008 (Angkavanish *et al.*, 2009). Colic is one of the GI problems in captive elephant which could be found in all age, especially in geriatric elephants. The older elephants were found colic quite often due to lower of digestible abilities. The partial caused of colic is improper feeding management such as type of roughage, massive indigestible diet in short period, and soil or dirt impaction. In Thailand, colic is classified as an emergency case for captive elephant, there are many elephants death because of colic every year. The clinical signs as same as in equine, include frequent lying down and getting up, non-appetite, lack of defecation, and abdominal distension (Rose and Rose, 1988). For the first aid or conservative treatment, the mahouts always stimulated defecation by rectal massage, water enema, giving the tamarind, and taking their elephants walk. Moreover, the treatment which veterinarians in Thailand commonly used was the combination of conservative and contemporary treatment like analgesic drugs, antibiotics, GI stimulants along with fluid therapy. However, it was not success in every case. Ten percent of the colic cases were lead to death (unpublished data). In equine, cecal trocarization was the treatment of choice in the severe abdominal distention cases which can be life-threatening condition (Fowler and Mikota, 2006). The purpose of gas decompression is to relieve the pain, improve the cardiovascular and respiratory status of the animal, and stimulate cecal motility. The aim of this report is to descript the procedure of the cecal trocarization in captive Asian elephant as an emergency procedure.

**Materials & Methods:** Fifty years old female Asian elephant showed colic-like clinical signs such as both-side abdominal distension, non-appetite, lack of defecation, and not drinking water for 24 hours. The veterinarians had treated both conservative and contemporary treatment for 2 days. The fluid therapy and medication agents were administered such as flunixin meglumine (2.2 mg/kg), simethicone (0.6 mg/kg) plus activated charcoal (1.7 mg/kg), enrofloxacin (3 mg/kg), dexametazone (0.4 mg/kg), and etc. The veterinary team made a decision to perform cecal trocarization procedure in this case to save her life from severe condition as respiratory discomfort from abdominal pressure by gas accumulation in bowel. This condition was determined by the lower of respiratory rate, change of the mucous membrane color and pulsation weakness, palpated at the ears. Moreover, gasping was found.

The procedure of cecal trocarization in Asian elephant could be explained as following. First, the trocarization site was located at the area in right paralumbar fossa where the skin was most flexible when palpated, or at the area where ping sound was detected. Second, this area was scrubbed with 1% chlorhexidine scrub, 70% alcohol, and 10% povidone iodine solution, respectively. Third, the fan-shaped superficial local anesthesia technique with underlying muscle with 10% lidocaine were performed using needle 18G 1.5 inches long. After 10-15 minutes, the skin was stabbed with sterile surgical blade for the width equal to the trocar's diameter. Fourth, the 4 inches trocar and the cannula were inserted through the skin layer, muscle,

peritoneum, but not cecal wall. After, the cannula was removed, the 6 inches spinal needle connected with syringe was inserted through trocar to penetrate the cecal wall to release gas from cecum. The disinfectant agent, povidone iodine solution was poured via spinal needle into cecal lumen in order to eliminate the bacteria which produced gas. Final step, wound was closed with sterile gauze. The elephant was monitored for at least 30 minutes. In this process, not only the vital sign should be detected such as respiratory rate, respiratory pattern, pulse, and color of mucous membrane, but also other signs of pain and the recurrent of abdominal distension were also important.

**Results:** After the cecal trocarization was performed, the elephant showed clinical signs which indicated the pain relief; for instance, no gasping, normal of color of mucous membrane, and normal respiratory rate were detected.

**Discussion:** Accordingly, the cecal trocarization technique should be considered as one procedure that enhanced the respiratory and blood supply in case of severe abdominal distension in elephant. Therefore, the equipments of cecal trocarization should be specifically designed for elephant such as the trocar-cannula length should be approximately 10-12 inches, and increase of diameter up to 0.5 inch. for suitable in release gas. The decision to do the cecal trocarization depends on various factors like poor respiratory condition or the abdominal distension as life-threatening, negative response to analgesic drug, the availability of equipment and medical agents, and permission of elephant's owner. Moreover, the standard operation procedure (SOP) of the emergency case like this should be planned in advance.

**Acknowledge:** We would like to thank the owner of Mae Sa Elephant Camp for their permission to treat and perform necropsy this elephant. Even the elephant was not survived but her case was memorable and invaluable to veterinarians, veterinary students, and mahouts.

**Key words:** Asian elephant, cecum, trocarization, gas, decompression

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### Genetic Relatedness and Behaviours of Releasing Asian Elephants (*Elephas maximus*) in the Elephant Reintroduction Project

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#### Introduction:

Elephants in the reintroduction project were recruited from several parts of Thailand, and their behaviors, i.e. social organization, differed individually. Previous reports illustrated that wild elephants with the matriarch in the herd contained the same matrilineal line and had close genetic relatedness (Fernando and Lande, 2000; Archie et al., 2006). We, therefore, hypothesized that the reintroduced elephants would set up the social structure based on maternal inherited DNA and genetic relatedness. The objective of this study was to investigate the genetic relatedness and behavioral relationship of the elephants in the reintroduction project.

#### Materials & Methods:

Information of social bonding behaviors was collected weekly and data analyzed for 12 months. Blood samples were collected from 65 elephants before releasing them to the Sublanka and Doiphamuang Wildlife Sanctuary, and DNA was extracted using 12 microsatellite loci (FH60, FH94, LafMS03, LafMS05, LafMS06, LafMS08, LA2, EMU04, EMU07, EMU14, EMU15, and EMU17 (Comstock et al., 2000; Eggert et al., 2000; Nyakaana and Arctander, 1998; Nyakanaa et al., 2005; Kongrit et al., 2008). A 600-bp fragment of the control region was amplified and sequenced using the primers MDL5 and MDL3, as described by Fernando et al. (2000).

#### Results:

There were 4 elephant groups (range 3-10 individuals/ group), and 5 isolated elephants in Sublanka Wildlife Sanctuary; and 6 elephant groups (range 2-5 individuals/ group), and 9 isolated elephants in Doiphamuang Wildlife Sanctuary. The results showed the average pairing relatedness of the elephants in the same group was 0.075, while that of isolated elephants was 0.038. Several mitochondrial haplotypes were found in the same elephant group. Furthermore, the observed heterozygosity in the Sublanka and Doiphamuang wildlife sanctuaries were 0.7385 (n = 29) and 0.8083 (n = 36) respectively, and 12 mitochondrial haplotypes were found in each location.

#### Discussion:

It appears that social bonding of the reintroduced elephants was not influenced by genetic relatedness and mitochondrial DNA haplotype, but was impacted more by the elephant calf, which was at the center of gatherings. The genetic unrelated social organization from our study was the same as the report in heavily poached African populations in which the long tusk old females or matriarchs were poached, and resulted in disrupting of the herd. The dispersed herd members; i.e. younger males and females, later formed a group without genetic relationship (Gobush et al., 2009), and from various mitochondrial haplotype (Nyakaana et al., 2001). Additionally, many elephants occasionally preferred isolation (Gobush et al., 2009). However, the management of reintroduction procedure should be performed by introducing the elephants, which carry closed pairwise relatedness and same mitochondrial haplotype, as this would increase the chance of group formation and lead to the establishment of naturally-linked elephant herd.

#### Acknowledgement

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#### Keywords:

Asian elephant, reintroduction, microsatellite, mitochondrial DNA, group forming behavior

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### Interferon-gamma Release Assay (IGRA) for the Diagnosis of Tuberculosis in Asian Elephants (*Elephas maximus*)

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**Introduction:** Tuberculosis (TB) is a re-emerging disease primarily caused by *Mycobacterium tuberculosis* in elephants (1). Culture of trunk wash is considered as a Gold Standard for TB diagnosis in the elephants; however, it has many limitations (2). Currently, DPP VetTB Assay (Chembio Inc., USA), a licensed test by United States Department of Agriculture (USDA), has been used in Nepal to diagnose TB in the elephants. A recent study showed that *M. tuberculosis* isolates from Nepalese elephants belonged to Indo-Oceanic Lineage which is also present in Nepalese TB infected human population suggesting that elephants might have contracted TB from the local residents and most probably from their handlers (3). As *M. tuberculosis* is an intra-cellular bacteria, so cell-mediated immunity is elicited by the body at the early stage of the infection for the protection. Among different cytokines, interferon- $\gamma$  is a major mediator involved in the immune-pathogenesis of tuberculosis (4).

**Materials & Methods:** The Interferon-gamma Release Assay (IGRA) was carried out using sandwich ELISA in 40 Asian elephants from three protected areas of Nepal. The IGRA developed and optimized for the diagnosis of TB in Asian elephants was used for this purpose. The best combination was obtained when capture antibodies and detection antibodies were used at the concentration of 1  $\mu$ g/ml and 3  $\mu$ g/ml respectively. The ELISA system developed was thus able to detect the native elephant interferon- $\gamma$ . The anti-elephant IFN- $\gamma$  rabbit polyclonal antibodies were used for both coating and detection antibodies, however, the detection antibodies were biotinylated. The assay was performed in 4 DPP VetTB Assay positive, 22 DPP VetTB Assay negative, 5 on-treatment and 9 treatment-completed elephants. The whole blood was collected and the stimulation of the blood was done in NIL, TB Antigen (ESAT-6/CFP-10), Phytohemagglutinin (PHA), Pokweed Mitogen (PWM) and Phorbol Myristate Acetate/Ionomycin (PMA/I). The whole blood collected was incubated for 16-24 hours at 37°C. The sandwich ELISA was performed on the harvested plasma samples using the sandwich ELISA at the Optical Density of 450nm. The OD values were converted to IU/ml and the values of IFN-gamma were compared among different population of elephants.

**Results:** The results showed that mitogenic stimulation by Phorbol Myristate Acetate/Ionomycin (PMA/I) was highest followed by Pokweed Mitogen (PWM) and Phytohemagglutinin (PHA). The sandwich ELISA system developed was thus able to detect native interferon- $\gamma$  from the elephants. The IU/ml values of interferon- $\gamma$  in all DPP positive values were higher than 0 IU/ml and the values in three DPP positive elephants are higher than the human cut-off values (0.35 IU/ml). So, these findings suggest that the IGRA has a greater potential as a diagnostic test for the tuberculosis in Asian elephants.

**Discussion:** The IGRA was thus able to detect the native elephant interferon- $\gamma$  in sandwich ELISA. The results showed that IGRA has the potential for the early diagnosis of TB in Asian elephants. The IGRA studying large population of Asian elephants with inclusion of more tuberculosis specific antigens will help to diagnose TB in early stage of disease. This will definitely help to start treatment soon, which will lead to prevention of transmission of tuberculosis to their handlers and other susceptible hosts. In the long run, this will eventually help in the conservation of Asian elephants.

**Keywords:** Asian elephants, Interferon- $\gamma$ , Sandwich ELISA, Tuberculosis

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### ASZWM-WDA JOINT SESSION FOR ASIAN WILDLIFE DISEASES SURVEILLANCE: Rabies in Wild Animals

#### Future Collaboration between ASZWM and WDA for Wildlife Diseases Surveillance

Nabin Rayamajhi, Tokuma Yanai, Junpei Kimura and Manabu Onuma  
ASZWM Board

Based on previous open discussion on "How to collaborate with Wildlife Diseases Association (WDA) to establish the wildlife diseases surveillance system in Asia by ASZWM" in the last ASZWM meeting in Singapore, we ASZWM boards started to contact with Wildlife Diseases Association (WDA). As a first step, ASZWM boards sent Dr. Yanai and Dr. Nabin to the 69<sup>th</sup> Annual Meeting of WDA held in New Mexico from July 26 to August 2nd. As a representative of ASZWM, Dr. Yanai and Dr. Nabin joined the WDA council meeting on July 28 and made a presentation entitled "Activity of ASZWM for Asian Conservation Medicine". At the council meeting we had discussion on future collaboration between ASZWM and WDA. During the meeting we also had an opportunity to discussion with the official members of WDA, WDA President Dr. Thierry Work, Dr. Dave Jessup, and the next president Dr. Marcela Uhart, as well as our adviser Dr. Pam Whitely and Dr. Hongxuan He from China. After some discussion, we made an agreement for future collaboration. Also, we agreed to start discussion for MOA to reinforce relationship for further collaboration between ASZWM and WDA. Hopefully we expect to have some common ground to start with by next WDA meeting in Australia.

At conference, Dr. Nabin made a presentation entitled "A new network for "Asian Conservation Medicine" to let participants know what we have done for Asian Conservation Medicine. Dr. Yanai presented on EHV-9, the original study from Asia. WDA council members and participants expressed their interest in our Asian society for some future collaboration and mutual benefit.



At WDA council meeting (left).



Discussion with WDA officers (right)



## Discovery of Rabies in a Wild Carnivore in Taiwan

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**Abstract:** During 2012–2013, an outbreak of rabies virus (RABV) infection occurred among ferret badgers in Taiwan. The study of 3 initial dead cases reveals the pathological lesions cover the characteristics of RABV infection in the animals. From the sequencing study, the virus strain is a distinct lineage and could intrude into Taiwan 91–113 years ago which suggests the virus could be cryptically circulating in the environment. Although rabies is possibly one of the oldest zoonotic diseases, the outbreak of the disease in Taiwan represents the complex interaction between RABV and its host, and lots of them still need to be study.

**Key Words:** Rabies Virus (RABV), Ferret Badgers, Phylogenetic Analysis, Diagnosis, Taiwan.

**Introduction:** After the last reported cases of rabies in a human in 1959 and a nonhuman animal in 1961, Taiwan was considered free from rabies. However, during 2012–2013, an outbreak occurred among ferret badgers in Taiwan. In the present study, diagnosis of the initial cases in the early outbreak and attempt to find the origin of the rabies virus (RABV) through phylogenetic analysis were unveiled.

**Material and Methods:** During May 2012–January 2013, through a government-supported program of routine disease surveillance of free-range dead wild animals that had been killed by vehicles or were receiving treatment for injuries and/or illness at the wildlife first aid station, 3 dead Taiwan ferret badgers (TWFB; *M. moschata subaurantiaca*) were submitted to the School of Veterinary Medicine, National Taiwan University, for further examination. A routine pathological examination was performed. After finding the unusual CNS lesions, molecular diagnosis and phylogeographic analysis were subsequently performed. IFA and Immunohistochemistry were included in the confirmation of the cases.

**Results:** Pathologic examination revealed nonsuppurative meningoencephalomyelitis with formation of eosinophilic intracytoplasmic inclusion bodies in all 3 animals. Reverse transcription PCR and immunohistochemical staining excluded the possibility of infection with the canine distemper virus. However, the results of fluorescence antibody testing, immunohistochemical staining, and reverse transcription PCR, followed by sequencing for RABV, were positive. In order to find out the origin of the virus, multiple sequencing of the N and G genes and of the complete genome, phylogeographic and molecular clock analyses were performed. The results demonstrated that the RABV affecting the Taiwan ferret badgers (RABV-TWFB) is a distinct lineage within the group of lineages from Asia and that it has been differentiated from its closest lineages, China I (including isolates from Chinese ferret badgers) and the Philippines, 158–210 years ago. The most recent common ancestor of RABV-TWFB originated 91–113 years ago.

**Discussion:** Our findings indicate that RABV infection is diagnosed in ferret badger, the wild carnivore, in Taiwan. The pathological lesions of RABV infection in the ferret badgers cover the characteristics of RABV infection in the animals. In order to optimize the diagnostic accuracy; however, the lesion and viral load distributions in different compartment of the brain tissue still need to be clarified. From the sequencing study, the virus strain is a distinct lineage and could intrude into Taiwan 91–113 years ago which suggests the virus could be cryptically circulating in the environment. Till now, RABV infection seems only limit in the ferret badger in this outbreak. Only one shrew and one dog were reported in this outbreak and the ferret badger-bited was clearly related to the dog case. Although rabies is possibly one of the oldest zoonotic diseases, the outbreak of the disease in Taiwan represents the complex interaction between RABV and its host, and lots of them still need to be studied.

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## The Zombie Ferret-badger: Epidemiology and Impact of Rabies on Ferret-badger Population in the Coastal Mountain Range, a “Hotspot” Enzootic Area in Taiwan

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**Abstract :** In 2013, rabies outbreak was confirmed in Taiwan with small-toothed ferret-badger as the primary suspected reservoir. This study, conducted in the Coastal Mountain Range, aimed to understand the epidemiology of rabies and its effects to the ferret-badger population. Result showed a severe impact of rabies on the local ferret-badger population and it is questionable whether the virus could still be circulated in the area with extremely low ferret-badger density. Evidences also suggested that other undiscovered rabies host species, such as forest dwelling bats, might exist. Further surveillance, therefore, should cover a broader range of wildlife species.

**Key words:** rabies, ferret-badger, population impact, reservoir.

**Introduction :** Endemic rabies had never been found since 1961 in Taiwan. In July, 2013, one small-toothed ferret-badger (*Melogale moschata subaurantiaca*) from Nantou County, central Taiwan was confirmed positive with rabies virus infection. The rabies surveillance program and retrospect study on the archived samples revealed the circulating of rabies virus in ferret-badger population. The earliest case could be tracked back to July, 2010. Until September 11, 2014, over 4,000 individuals of different wild and domestic species were screened for rabies infection, and 387 (out of 995) ferret-badgers, 1 domestic dog and 1 Asian house shrew (*Suncus murinus*) were found positive. Phylogenetic analyses on the rabies strain from Taiwan suggested the isolation of Taiwan ferret-badger strain (TWFB) from its closest lineages (China I and Philippine strain) for over 100 years. This result indicated the cryptic circulation of the TWFB in Taiwan in the past. Although ferret-badger was found to be the principle host for TWFB, the epidemiology and effect of rabies in ferret-badger population was still remained unknown. We conducted the research for understanding the epidemiology and population impact of rabies on wild carnivores and identifying the reservoir for the re-emerged wildlife rabies in the southern part of the Coastal Mountain Range, eastern Taiwan, a “hotspot” of rabies in Taiwan.

**Materials & methods:** Small carnivores trapping, population monitoring by camera trappers, and resident interview were conducted in Luanshan and Chenggong area, Taitung County. Those two areas were found with high rabies cases of ferret-badger, compared with other parts in Taiwan.

**Results:** A total of 3 masked palm civets, 27 crab-eating mongooses and 14 ferret-badgers were trapped. Nine ferret-badgers were died after trapped, and all of them were found positive with fluorescent-antibody test that gave a 64% (=9/14) infectious rate and a 100% case fatality rate. Four died individuals were tested for antibody, with 3 showed positive result. The 5 survived ferret-badgers and all 3 masked palm civets and 27 crab-eating mongooses showed negative with antibody screening. Population monitoring revealed that the abundance of the ferret-badger in Luanshan and Chenggong experienced a more than 90% and 80% decreasing after the outbreak of the TWFB, respectively. Local residents interview indicated that this massive mortality of ferret-badger has never seen before.

**Discussion:** Severe population impact of rabies on ferret-badger population was found and it is questionable whether the TWFB could still be circulated in the area with such a low ferret-badger density after outbreak. The evidences, included high case-fatality rate, severe population impact, first massive mortality recorded in the study area, and the result of phylogenetic analysis (Chiou et al. 2014), indicated that the exist of other reservoir species could not be rule out. Therefore, other than continue active carnivore surveillance programs in different enzootic area, further surveillance should also include forest dwelling bats (Leslie et al. 2006), in order to clarify their possible role in rabies transmission in Taiwan.

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## Rabies Control and Vaccine Development for Wildlife in Korea

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**Introduction:** Rabies is an important viral zoonosis with acute encephalitis and death in mammals. Rabies causes approximately 60,000 human deaths worldwide per year, mostly in Asia and Africa. Rabies virus (RABV), a causative agent of rabies, is a member of the *Lyssavirus* genus of *Rhabdoviridae* family. RABV has approximately 12-kb of unsegmented negative-strand RNA for encoding the genes of the nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and polymerase (L). The glycoprotein is significantly important for attachment of the virus to the cell, pathogenicity, immunogenicity, and neurovirulence. Many recombinant vaccines are based on glycoprotein because this protein is considered as the main antigen that induces neutralizing antibodies. Although several rabies vaccines are developed and available, urban rabies is still serious public health problems in many developing countries, and sylvatic rabies remains in wild animals of many developed countries. In Korea, first outbreak of rabies was in 1907. No animal rabies case was reported between 1985 and 1992 with effective RABV control program. Since rabies reoccurred in 1993, rabies cases have been reported in Gyeonggi and Gangwon province annually. The current main reservoir transmitting to other animals such as cattle, dogs, and cats is the raccoon dogs (*Nyctereutes Procyonoides*) in Korea. To achieve rabies control in Korea, massive vaccinations were conducted for domestic animals and oral bait vaccine were distributed to wildlife. In this study, we report the construction of recombinant human adenoviruses expressing the RABV G and N protein and evaluation of protective immunity of combination of recombinants in mice after challenge with RABV to develop prospective rabies vaccine strategy for domestic animals and wildlife animals in Korea.

**Materials & Methods:** Recombinant human adenoviruses expressing glycoprotein (G), ectodomain plus signal peptide (SP-ED) of G, and nucleoprotein (N) of Korean rabies strain (KRVB0910) were constructed using the ViraPower™ Adenoviral expression system (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The generated recombinant adenoviruses Ad-Gfull, Ad-Gsped and Ad-Nfull were identified by electron microscopy (EM), immunofluorescence assay (IFA) and Western blot. In order to assess the protection of recombinant adenoviruses expressing sped of G protein (Ad-Gsped) and complete G protein (Ad-Gfull) in combination with recombinant adenovirus expressing N protein (Ad-Nfull) against challenge with a lethal RABV, fifty BALB/c mice were randomly grouped into 5 groups, immunized intramuscularly with recombinant adenoviruses and challenged with RABV CVS-N2c. In addition, mice were immunized with Raboral VRG bait vaccine and mixed virus (Ad-Gfull + Ad-Nfull) by oral route and challenged with RABV CVS-N2c. After challenge, body weight and survival of mice was daily checked for 2-3 weeks.

**Results:** Typical icosahedral capsids of adenovirus were observed in the 293A cells infected with all recombinant adenoviruses by EM. The expression of sped of G protein, complete G and N protein of RABV was analyzed by IFA and Western blot. Intramuscularly immunized mice of all groups conferred complete (100%) protection against the RABV in intramuscular challenge. In the intracranial challenge, only mice immunized with mixed viruses of Ad-Gfull and Ad-Nfull showed 100% protection. Also, mice were immunized with Raboral VRG bait vaccine and mixed virus (Ad-Gfull + Ad-Nfull) by oral route had complete (100%) protection against the RABV.

**Discussion:** In this study, we constructed recombinant human adenoviruses expressing glycoprotein (G), ectodomain plus signal peptide (SP-ED) of G, and nucleoprotein (N) of Korean rabies strain (KRVB0910) and evaluated that intramuscular and oral immunization with mixed virus (Ad-Gfull + Ad-Nfull) confer complete protection against RABV in mice. Further study is in progress to evaluate the immunogenicity of recombinant adenoviruses in the raccoon dogs. Recombinant adenoviruses of this study are expected to be a promising candidate of new rabies vaccine for wildlife animals in Korea.

**Keywords:** rabies, wildlife, vaccine

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## Status of Oral Rabies Vaccination in Wild Animals in the Seoul, Metropolitan Area, Korea

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Rabies is one of the most fatal zoonosis. Rabies eradication programs have been established and implemented in many countries in order to eliminate the disease. The most significant targets for oral rabies vaccination (ORV) programs are wild animals, mainly wild carnivores. Raccoon dogs (*Nyctereutes procyonoides*) are the most important hosts in Korea. From 1985 to 1992, there was no outbreak report of rabies in Korea. However, rabies re-occurred in 1993. Since then, rabies had been occurred only the northern part of Gyeonggi and Gangwon provinces, particularly north of Han River. In September 2006, rabies occurred in the Seoul Metropolitan City. Consequently, the municipal government of Seoul decided to implement ORV programs. From 2006 to 2012, ORV was implemented along the northern border between Seoul Metropolitan City and Gyeonggi province, particularly north of Han River. However, in 2013, rabies outbreak was newly reported in Hwaseong city in Gyeonggi province which is located south of Seoul and south of Han River. Thus, ORV expanded along the southern border of Seoul Metropolitan City. Currently, ORV is implemented along all of the border of Seoul biannually (March and November). The containment barrier is approximately 115 km long and 40,000 doses are distributed throughout the barrier. 30 doses are distributed every 100 meters by hand. Using GPS (Global Positioning System) module, locations of distribution are geotagged and analyzed.

Key word: Rabies virus, Oral rabies vaccination, Seoul Metropolitan City, Raccoon dog

## Rabies in an Infant Orangutan (*Pongo pygmaeus*) at Borneo Orangutan Survival Foundation (BOSF) Nyaru Menteng

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**Abstract:** We present a report of what we believe to be the first known case of rabies infection in an orangutan. A nine-month-old male infant orangutan (*Pongo pygmaeus*) was confiscated from illegal private ownership in a village in Central Kalimantan, Indonesia. Clinical signs began five days after his arrival, and included mild fever, poor skin turgor, hyper-salivation, restlessness, aggression, screaming and intermittent nystagmus and loss of consciousness. Treatment was unsuccessful and the orangutan died six days after the onset of symptoms. Necropsy revealed extensive cerebral inflammation; rabies was confirmed by fluorescent antibody technique (FAT).

**Key Words:** Fluorescent Antibody Technique, Orangutan, Rabies, Zoonosis

**Introduction:** Rabies is a fatal and acute encephalomyelitis disease caused by viruses of the *rhabdoviridae* family; it is known to affect many mammalian species including humans, and thus is an important zoonotic disease. We present here a report of what we believe to be the first known case of rabies infection in an orangutan.

**Materials & Methods:** A nine-month-old male infant orangutan (*Pongo pygmaeus*) was confiscated from illegal private ownership in a village in Central Kalimantan, Indonesia, on 24<sup>th</sup> October 2013; he was handed over to the Borneo Orangutan Survival Foundation at Nyaru Menteng, Central Kalimantan, Indonesia, where he joined a quarantine group.

**Results:** Clinical signs began five days after his arrival, and included mild fever, poor skin turgor, goose-bumps, hyper-salivation, restlessness, aggression and screaming, with a tendency to bite; there was also intermittent nystagmus and loss of consciousness. Tests for helminths, malaria and typhoid were negative and broad cover treatment was administered including antibiotics, anti-tetanus serum, antimalarial, anti-inflammatory and anti-pyretic drugs. Treatment was unsuccessful and the orangutan died on 4<sup>th</sup> November 2013, six days after the onset of symptoms. Necropsy revealed extensive cerebral inflammation; rabies was confirmed by fluorescent antibody technique (FAT) testing on the hippocampus.

**Discussion:** It is almost certain that this orangutan acquired rabies before arrival at the center; Kalimantan is still endemic for rabies amongst the dog population. This case was isolated from other orangutans at the onset of symptoms, and an extended quarantine for all in-contact animals has been completed with no evidence of spread. All people in contact with the case received post-exposure prophylaxis.

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### The Role of Animal Welfare in the Health Husbandry and Conservation of Zoo Species

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#### Introduction:

Wild animals are under threat from habitat loss, poaching and trade. Zoos should be a key stakeholder in delivering effective conservation and zoo animal health and husbandry programmes. But in some countries, especially in regions such as Eastern Europe and Asia, zoos may actually act as consumers of endangered wildlife, often because of poor animal reproduction and high mortality rates in the captive setting. In many cases, causes of poor reproductive rates, injury, illness and high mortality, can be linked to poor standards of zoo animal health, husbandry and welfare.

Animal use in zoos is controversial and may be criticised unless the welfare of animals is guaranteed (Wickins-Dražilová, 2006, BornFree, 2012). Access to animal welfare and husbandry training materials has historically been limited in the global zoo community (Waugh and Wemmer, 1994, Goulart et al., 2009) and may be limited by the English language bias (Goulart et al., 2009). Training in animal welfare is important because of its impacts on animal health, international trade, industry, economic viability, and consumer perceptions (Tadich et al., 2010).

The lack of animal welfare training in zoos has inhibited a preventative medicine approach to animal welfare problems, with insufficient welfare indicators for zoo animals developed (Wickins-Dražilová, 2006) and zoos taking remedial action only once poor welfare is identified (Melfi, 2009). This necessarily requires animal suffering to exist before action is taken.

#### Materials and Methods:

This presentation will outline the differences between ethical constructs and animal welfare science, and discuss the responsibilities of the zoo vet in terms of animal health and welfare, which may sometimes conflict with personal ethics or conservation goals. It will describe current developments in zoo animal welfare around the world, including in Europe, China and the USA (DGSanco, 2011, FVE-AVMA, 2011, Whitham and Wielebnowski, 2013).

#### Results:

Significant political and cultural limitations relating to the open discussion and critical evaluation of animal welfare still exist around the world, both within the zoo community and externally, impeding the development and promotion of good zoo animal welfare. However, through international partnerships and collaboration, significant progress in zoo animal health, welfare and conservation can be made.

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## INFECTIOUS DISEASE

### Opportunistic and Pathogenic Mycobacteria: Importance of Systematic Surveillance of Mycobacteriosis based on Pathological Diagnosis

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#### Abstract

Genus *Mycobacterium* lives in natural environments such as soil, water, and surfaces on animals and plants. Some species can infect animals as pathogens, which cause tumours and granuloma on skins and various organs. In contrast to great efforts against virulent species such as *M. tuberculosis* complex, putative virulent mycobacteria, categorized as 'opportunists', have not been paid attention to for the purpose of understanding their actual situation such as natural niche, reservoirs, and transmission routes. Systematic surveillance of mycobacteriosis cases based on pathology and recent

development on comparative genomics may shed light on unsureness of the nature of mycobacterial species as pathogens. To launch the project, we established a network named ASZWM mycobacteriosis surveillance group. The network consisted of people from several Asian and Oceanic countries, which might be the first network in ASZWM for wildlife diseases surveillance in Asia.

#### Results and Discussion:

Genus *Mycobacteria* includes more than 30 pathogenic species to humans. Besides strict pathogens such as *M. tuberculosis* complex, *M. leprae*, and *M. ulcerans*, opportunistic infection of other mycobacterial species with immune-deficient hosts have become to be considered as important cases. Not only humans but also various animals are often infected with such mycobacteria, however, their origins of infection, transmission routes, and existence or absence of natural hosts have remained unknown.

Recently, we experienced mycobacteriosis cases of various animals having in zoos. (1) *M. genavense* from diamond doves. In this case, two doves were found dead by mycobacteriosis. Fecal samples of the remaining doves in the same flock were positive for mycobacterial infection, and thus they were euthanatized (Haridy et al. 2014). (2) *M. kansasii* from black bared sakis (new world monkeys). Two monkeys in a same cage were infected with the bacilli in 2009. One was died with severe symptom of mycobacteriosis (Murai et al. 2012). The other had repetitious symptoms and resected twice to remove infected parts. (3) *M. marinum* from green tree frogs. In 2013, one frog died from mycobacteriosis. One week later, other three frogs in the same tank presented with ulcerative and nodular cutaneous lesions on the brisket, limbs, fingers, and ventral abdominal surface (Haridy et al. 2014). The etiological mycobacteria were determined by PCR amplification of specific genes of respective species or DNA sequencing of some genetic regions: 16SrDNA, internal transcribed spacer (ITS), *rpoB*, and *hsp65*. These sequences offer reliable information to determine mycobacterial species. They are also useful to draw phylogenetic trees when we want to determine novel mycobacterial species genetically.

Generally, once mycobacteriosis is diagnosed by pathological analysis, their species have not been determined in many cases. However, to understand ecological background of etiological mycobacteria and evaluate their risks, it is important to accumulate refined data of clinical cases as much as possible. To archive such information efficiently, we have proposed to analyse infected formalin-fixed specimens or paraffin blocks for pathological diagnosis, as a first step of the global collaborative trial. They are indeed useful resources to determine the species by purification and detection of mycobacterial DNAs. It is also important to isolate clinical strains from mycobacteriosis cases based on reliable pathology to separate etiological agents from free-living mycobacterial species. Live stocks of etiological strains can be used to determine their complete genome sequences immediately by next generation sequencers (NGS), which has been drastically developed recently. Systematic accumulation of genomic information of etiological mycobacteria may open a new era of mycobacterial research which has been at a standstill for a long time.

Understanding mycobacteria from a view of veterinary, medicine, and ecology will become a good model of 'One Health'. To launch the project, during previous meeting in Singapore and Kuching, we established a network named ASZWM mycobacteriosis surveillance group. The network consisted of people from several Asian and Oceanic countries such as Japan, Thailand, Nepal, Myanmar and Australia, which might be the first network in ASZWM for wildlife diseases surveillance in Asia. We expect that many investigators who have respective missions and interests can share the target as an international trial.

#### Key words:

Mycobacteriosis, mycobacteria, zoonosis, pathogenicity, One Health

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#### Monitoring of Outdoor and Zoonotic Infectious Diseases by using Hunting Dogs in Japan: Usefulness of Hunting Dogs as a Sentry

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**Introduction:** Hunting dogs are always wondering around bushes and forests to catch wild mammals and birds. They got a lot of chance to contact and bite with ticks as well as wildlife. To access risk of tick-born or wildlife-related infectious diseases including zoonosis, we are doing a serological survey by using serum of hunting dogs which were usually exposed to risks of infection from ticks or wild animals. This assessment may be useful to estimate risks for people who are working under then open sky.

**Materials and methods:** Total 5-6 cc of blood sample was collected from about 600 hunting dogs kept in 22 prefectures in all over Japan including Kyushu, Sikoku, Tyubu, Tohoku, and Hokkaido districts, as well as various islands and peninsula. The collected blood were centrifuged, and the serum were examined for various infections including zoonosis such as Lyme diseases (ELISA), leptospirosis (ELISA, MAT), *Corynebacterium ulcellans* (cell culture and toxin), Toxoplasmosis (ELISA) and *Francisella tularensis*. For tick-born canine protozoa *Babesia gibsoni* and *Hepatozoon canis* were examined by PCR.

**Results and conclusion:** Various positive rates were observed in diseases such as Borreliosis, Leptospirosis, *Corynebacterium ulcellans* and *Toxoplasma gondii*, which varied largely depending on the districts. There was no positive case in *Francisella tularensis* or tetanus. Of positive cases a large proportions were from cases collected in Kyushu districts in some infections, especially hepatozoonosis or canine filariosis. Based on these surveys, in some infections such as Borreliosis showed a correlation to the incidence and distribution of human patients, and it may be useful to monitor blood sample of hunting dogs in various districts to assess risks of outdoor tick-born or wildlife-related infectious diseases in humans. In order to analyze the spectrum of field zoonotic infections for a detailed hazard map of each region, it is necessary to increase the number of sample cases from hunting dogs.

**Key words:** hunting dogs, infectious diseases, monitoring

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#### Fatal Canine Distemper Virus Infection in Common Palm Civets (*Paradoxurus hermaphroditus*)

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**Introduction:** Canine distemper virus (CDV) causes severe disease in various species of carnivores. Morbidity and mortality vary greatly in different species. The case fatality rate of some species approaches 100% and is higher if there is neurologic involvement. Infected domestic dogs can act as asymptomatic carriers but fatal disease could generally occurs in canids, felids, hyaenids, mustelids, procyonids, ursids and viverrids. However, there is little information about CDV infection in viverrids particularly in common palm civet (*Paradoxurus hermaphroditus*). This study had recognized some clinical aspects from an outbreak of Canine distemper in common palm civet farm in Thailand.

**Materials & Methods:** Apart from outbreak investigation, clinical manifestation was assessed in 24 from total 38 common palm civets in the farm. The animals were observed and examined for clinical signs and symptoms. Rapid screening test was conducted in order to initiate diagnosis and molecular

technique was done for confirmation. Clinical manipulation, control and prevention measures were implemented in order to limit disease spreading and animal loss.

**Results:** Of 24 civets, 23 (96%) were inappetent and markedly anorectic. Integumentary abnormality was observed in 20 (83%) civets noticeably hard pads. Purulent oculonasal discharge and sneezing suggested upper airway inflammation, and secondary bacterial infection in 16 (67%) and 4 (17%) civets respectively. 6 (25%) of observed animals represented respiratory distress, for instance dull lung sound, hyperpnea and dyspnea. Gastro-intestinal impairment including watery and bloody diarrhea was recognized in 38% of these civets. Nearly half of all animals observed showed neurological symptoms such as ataxia, seizure, spastic paralysis and cycling. Supportive and conjunctive treatments were ineffective and not favorable so 22 of 24 civets died making case fatality rate as high as 92%.

**Discussion:** Mild illness, with nonspecific signs and symptoms like anorexia, fever and upper respiratory tract infection, may occur in domestic dogs. Acute generalized canine distemper is related to the respiratory and gastro-intestinal systems and includes conjunctivitis, pneumonia and diarrhea (often haemorrhagic). As in this outbreak, the neurologic manifestation usually occurs 1-3 week after recovery from acute infection. Only two viverrid species were documented regarding canine distemper namely binturong (*Arctictis binturong*) and masked palm civet (*Paguma larvata*). Clinical signs includes dehydration, dyspnea, serous oculonasal discharge, diarrhea, and convulsion. Treatment was dissatisfactory thus prevention protocol such as immunization is strongly recommended.

**Keywords:** Canine distemper virus, Viverrids, Common palm civet, clinical manifestation

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### A Relapsing Fever Group *Borrelia* sp. similar to *Borrelia lonestari* found among Wild Sika Deer (*Cervus nippon yesoensis*) and *Haemaphysalis* spp. ticks in Hokkaido, Japan

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5. Presentor

#### Abstract

A relapsing fever group borrelia similar to *Borrelia lonestari* was detected from sika deer and *Haemaphysalis* ticks in the eastern part of Hokkaido. In total, this *Borrelia* sp. were found in 11.2 % of deer samples using PCR. The prevalence was significantly higher in fawns than in adults ( $P<0.05$ ) and was higher in Shiretoko region than in Shibetsu ( $P<0.01$ ). In Shiretoko, *Haemaphysalis* spp. were more abundant than *Ixodes* spp., on the deer and in the vegetation but the rate were opposite in Shibetsu. Using real-time PCR, this borrelia was detected from 2/290 adult and 4/76 pools of nymph *Haemaphysalis* tick.

**Keywords:** *Borrelia lonestari*-like, *Haemaphysalis* spp., sika deer, Hokkaido

#### Introduction

The genus *Borrelia* is comprised of three phylogenetic groups: Lyme disease (LD) borreliae, Relapsing fever (RF) borreliae, and Reptile-associated (REP) borreliae. LD and REP borreliae are transmitted by ixodid (hard-bodied) ticks while most RF borreliae are transmitted by argasid (soft-bodied) ticks, with several exceptions as; *Borrelia recurrentis* infected by lice, and *Borrelia theileri*, *Borrelia miyamotoi*, and *Borrelia lonestari* use hard-bodied ticks as vectors. *B. theileri* is the causative agent of bovine theileriosis and *B. miyamotoi* was recently reported the human pathogenicity. But, the pathogenicity of *B. lonestari* is still unclear after the first report of 1995 in USA. Understanding the biology of these hard-tick transmitting RF borreliae has advanced slowly due to the difficulty of cultivation. In the United States, the white-tailed deer and *Amblyomma americanum* tick are considered as the main reservoir and the vector of *B. lonestari*. There have been two reports of *Borrelia* spp. close to *B. lonestari* outside of the United States; a RF *Borrelia* sp. close to *B. lonestari* and *B. theileri* in Brazil and another RF *Borrelia* sp. from the *Amblyomma geomydae* in Okinawa, Japan, of which sequences was clustered with *B. lonestari* and *B. miyamotoi* in phylogenetic analysis. These findings suggested the possibility that unknown *Borrelia* spp. exist worldwide. In a previous survey, borrelial DNA fragments similar to *B. lonestari* (herein referred to as *B. lonestari*-like) were found among blood samples from sika deer (*Cervus nippon yesoensis*) in Hokkaido. To understand how this borrelial organism is maintained in the ecosystem, we conducted surveillance of wild sika deer and ticks in Hokkaido, Japan.

#### Materials and Methods

We sampled deer from hunting and nuisance control culling held in the eastern part of Hokkaido from July 2011 to August 2013. Deer were grouped by sampling region (Shiretoko and Shibetsu), age (fawns and adults at 1 yr old), sex (female and male) and season (winter and summer by snow covering period on vegetation). Blood were sampled for the molecular survey and a portion of an entire ear, when possible, were collected and kept at  $-20^{\circ}\text{C}$  for attached tick survey. From May through September of 2012 and 2013, questing ticks on vegetation were collected by flagging in Shiretoko and Shibetsu and were identified to species and stage. DNA was extracted from deer blood and *Haemaphysalis* ticks from vegetation. All deer blood DNA samples and tick DNA were examined using nested PCR to detect the *Borrelia* spp. flagellin gene (*flaB*) and real-time PCR to detect the 16S rRNA gene of *B. lonestari*-like. The positive results in the nested PCR were sequenced for identifying its species. Only positive samples in both methods were counted as positive in the results. To define the genetic character of *B. lonestari*-like, 2 positive female tick samples were examined by PCR and sequencing for 16S rRNA gene (1,537 bp), and the *glpQ* (approx. 1.5kbp) with *flaB* (323 bp).

#### Results and discussions

In total, 224 blood samples were collected from sika deer and 25 samples (11.2%) were confirmed positive in both nested PCR and the real-time PCR. The infection rate of *B. lonestari* among White-tailed deer was reported about 8.7% in the United States and our result seems enough high to consider sika deer as the mammal reservoir of *B. lonestari*-like in Hokkaido. The infection rates of the two regions were significantly different in Shiretoko (22/123, 17.9%) and Shibetsu (3/101, 3.0%) ( $P<0.01$ , chi-square test). Additionally, in the tick populations in two regions, we could found more abundant *Haemaphysalis* spp. than *Ixodes* spp. in Shiretoko (736 *Haemaphysalis* spp. of total 940 collected ticks) and it was opposite in Shibetsu (32/573) ( $P<0.01$ , chi-square test) and this trend was also found in the deer-feeding ticks. As in the previous surveys of *Borrelia* spp. in *Ixodes* spp. ticks, *B. lonestari*-like have never been reported, we focused on *Haemaphysalis* spp. in this study. In these 768 host seeking *Haemaphysalis* ticks, 670 ticks including 290 adults and 380 nymphs were tested for *B. lonestari*-like using real-time PCR. Nymphs were pooled, with 5 heads in each pool. As a result, 2 *H. japonica* females and 4 nymph pools (2 pools of *Haemaphysalis megaspinosus*, 1 pool of *H. japonica*, and 1 pool of unidentified *Haemaphysalis* spp.) were positive. Compared to the prevalence of *B. lonestari* in *A. americanum* (2.5%), these rate were lower. Therefore, wider and further investigation in the vector ecology is necessary in the future. Additionally, the prevalence in fawns (7/32, 21.9%) was greater than twice of adults (18/192, 9.4%) ( $P<0.05$ , chi-square test). It may be due to the immune response of naïve fawn against the first exposure to this *Borrelia*. To understand the ecology of this unknown borrelia, serological research in the future will be essential. In logistic regression to compare four factors: age, region, season, and sex, region and age were confirmed to be the main risk factors for *B. lonestari*-like infection ( $P<0.01$ , Odds ratio (OR) 13.06, 95% Confidence Interval (CI) 6.99-24.03 and  $P<0.01$ , OR 4.23, 95% CI 2.38-7.53, respectively). The *flaB* sequencing of *B. lonestari*-like in this study was most similar to *Borrelia* sp. BR (Acc. No. EF141022) at 97% similarity using BLAST in GenBank. The 16S rRNA gene and *glpQ* were most similar to *B. miyamotoi* LB-2001 (Acc. No. CP006647,

at 99%) and *B. lonestari* MO2002-V1 (Acc. No. AY682922, at 92%), respectively. Phylogenetic trees were created for *flaB*, 16S rRNA gene, and *glpQ*. *B. lonestari*-like in this study consistently clustered with hard-tick vector RF borrelia group but distinguished with other borreliae in the cluster. As seen these results, *B. lonestari*-like may be the new member of this hard-tick vector RF group.

## Conclusion

Sika deer are potential mammal reservoir of the *B. lonestari*-like in Hokkaido. The higher molecular prevalence in fawn than in adult may be related with the immune response. And *Haemaphysalis* spp. ticks are the first candidate vector of this borrelia in Hokkaido. This is the first report of vector candidate and phylogenetic characters of the *B. lonestari*-like, newly reported RF group borrelia in Hokkaido.

## Surveillance of Avian Influenza Virus in Wild Bird Trade in Ha Noi during Spring Migration Season 2014

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**Abstract:** Wildlife trade and emerging infectious diseases are significant threats to both human and animal health. Legal and illegal trade in domestic and wild bird played an important role in the global spread of high pathogenic avian influenza (HPAI) H5N1 and other serious zoonotic diseases. Totally, 204 specimens representing 102 individuals were collected in three markets in Ha Noi during spring migration season 2014. The samples come from six species, belonging to three families of two orders. No HPAI viruses were detected; five low pathogenicity avian influenza (LPAI) viruses were isolated from three species, allocated 4.9%. A Chinese pond heron (*Ardeola bacchus*), two cattle egret (*Bubulcus ibis*) and two common moorhen (*Gallinula chloropus*) were positive for H6N6 LPAI viruses.

**Key words:** Avian influenza virus, wild bird, wild bird trade.

## Introduction

Wild bird, specifically species in these order Anseriformes and Charadriiformes are considered the natural reservoir of all 144 subtype of avian influenza virus. The risk that wild birds could move the AIVs, even the HPAI H5N1 subtype among countries and regions was from the illegal trade of wild and exotic birds. Wild bird trade provides disease transmission mechanisms at scale that not only cause human disease outbreaks but also threaten livestock, nature wildlife populations and the health of ecosystems. Therefore, the purpose of this study was to investigate the prevalence of avian influenza virus in wild birds in three markets in Ha Noi during spring migration season in 2014.

## Material and methods

- Sample collection: Cloacal and tracheal swab samples were collected from wild birds sold in market or on the road in sub-urban in Ha Noi. Specimens of each bird were stored in Viral transport medium (VTM).
- Virus isolation: Samples were inoculated individually into 9-10 day old embryonated chicken eggs. The allantoic fluid was harvested after two days incubation at 35°C and then at least two hours of inactivation at 4°C. Influenza A virus was detected using hemagglutination assay (HA) with chicken red blood cells. When HA test gave positive results, they were confirmed as type A influenza virus by Commercial influenza quick test.
- RNA extraction and RT reaction: RNA was extracted from allantoic fluid by QIAamp viral RNA mini kit (QIAGEN) according to the instruction from the manufacturer. cDNA, subsequently, was synthesized using RT-Uni 12 primer.
- Subtyping of avian influenza virus genes: HA and NA subtype of isolated virus was characterized by PCR with specific primer sets.

## Results

A total of 204 cloacal and tracheal samples from 102 birds was collected for avian influenza virus test. These birds came from six species, belonging to three families of two orders (Ciconiiformes and Gruiformes). The highest collected number was black-crowned night heron (*Nycticorax nycticorax*), occupying 40 birds (39.2%), followed by white-breasted waterhen (*Amaurornis phoenicurus*) and common moorhen (*Gallinula chloropus*), lying 21 birds (20.6%) and 20 birds (19.6%), respectively. The rest species allocated minus number, under 10 individuals.

There were five positive wild bird samples (4.9%) with avian influenza virus by both HA test and influenza quick test: a Chinese pond heron (*Ardeola bacchus*), two cattle egret (*Bubulcus ibis*) and two common moorhen (*Gallinula chloropus*). The positive rate of *Bubulcus ibis*, 28.6% (two out of seven), was the biggest in all surveillance species. The second and the third position belonged to *Ardeola bacchus* and *Gallinula chloropus*, staying 11.1% and 10% respectively. In contrast, although being highest in the number of sample, black-crowned night heron (*Nycticorax nycticorax*) has no positive sample. Similarly, the remaining species provided the same number of positive with *N. nycticorax*.

Interestingly, results of subtyping showed that all five positive strains were low pathogenic avian influenza virus H6N6; no high pathogenic avian influenza virus was detected.

## Discussion

The efforts to monitor wild birds have increased worldwide in recent years based on concern with the possibility that wild birds would disseminate the Asian H5N1 HPAIV. Vietnam in general and Ha Noi in particular provides a good location for wild bird monitor because there is on the route of migratory bird in the Pacific and Central Flyways. Besides, this research also provides an opportunity to collect additional data on the circulation of LPAIV in wild birds in Vietnam by contributing data to help establish the genetic relationships among wild bird origin avian influenza viruses.

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## Case Report of a Dyspneic Red-billed Hornbill parasitized by Cyclocoelid Trematodes in Jurong Bird Park, Singapore

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On January 2013, a female of the Red-billed Hornbill, *Tockus erythrorhynchus*, kept in Jurong Bird Park, Singapore, was admitted with severe dyspnea with high pitch sound. It vomited reddish fluid and was recumbent. It stood up for a few minutes after oxygenation but became unresponsive again and died shortly after. On the necropsy, many individuals of trematodes were obtained from air-sacs, lung and trachea. Based on the morphological



identification, it was identified with the genus *Szidatitrema*, (Cyclocoelidae). This is the first record of the cyclocoelid obtained from the Red-billed Hornbill. Taking the life cycle of the family Cyclocoelidae into consideration, the case may have swallowed snails contained the metacercaria of the trematodes. There has been no report on the pathological effect to the host bird by the trematodes, but the severe respiratory symptom might be provoked by the trematode parasitism.

The present study has been published on a journal, viz., *Jpn. J. Vet. Parasitol.*, **37**: 13-15, and was supported in part by the Supported Program for the Strategic Research Foundation at Private Universities (2013-2017) and a Grant-in-Aid (No. 26460513) of the Ministry of Education, Culture, Science and Technology, Japan.

## MARINE MAMMALS

### Cetaceans from Vietnamese Waters: A View from the Outside

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Until late in the 20<sup>th</sup> century, very few reports existed for cetaceans in Vietnam. Only five species were known: Irrawaddy dolphin, bottlenose dolphin, finless porpoise, pygmy sperm whale and blue whale. These records were mainly on stranded animals. At the close of the 20<sup>th</sup> century, Vietnamese and foreign scientists conducted preliminary surveys in "Whale Temples" and recorded skulls from sixteen species of cetaceans. Eleven of these had not been previously recorded. During October 1999 and April 2000, coastal and offshore surveys for cetaceans were conducted in the Gulf of Tonkin. Five species of small cetaceans were documented. Recent strandings of large whales from Vietnam will be discussed.

Aquariums in Vietnam which have or have held dolphins include: Oceanographic Institute of Nha Trang (Black Sea bottlenose dolphins); Tri Nguyen Aquarium, Nha Trang; Tuan Chau Aquarium, Ha Long Bay (*Tursiops* and *Orcaella*); Vinpearl Underwater World, Nha Trang; Vinpearl Aquarium, Times City, and Suoi Tien Tourist Area, Ho Chi Minh City; and Van Hien Wonderland, Dai Nam. Species held captive include at least *Tursiops* and *Orcaella*.

Museums/oceanographic institutes which have cetacean specimens include: the Institute of Oceanography, Nha Trang; Haiphong Institute of Oceanology and Research Institute of Marine Products, Haiphong; and the Yen Hung District Museum.

Needs for future research will also be discussed.

### Some Information about Marine Mammals in Vietnam

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#### Abstract

As a maritime nation, Sea waters areas of Vietnam are habitat and foraging for many different species, including marine mammals (whales, dolphins, dugong). However, in Vietnam at present there is no adequate statistics on the number, composition, and distribution of marine mammals groups, although each year has recorded the appearance of them in the region Vietnam's sea. Some were stranded, died and washed ashore through the public information. This paper was undertaken to gather the research on marine mammals in Vietnam, a collection of recent statistic on marine mammals agrounded and died in the coastal areas. In addition reports it also provides some information related to the storage of samples (bone whales, dolphins) of local residents to provide basic data on the status, distribution of marine mammals in Vietnam.

Key Words: Marine mammals in Vietnam, Temple of whales.

#### Introduction:

Vietnam has a coastline of 3,350 km, stretching over 1 million km<sup>2</sup> of sea areas, the total number of small islands and reefs around 2,800. With the vast of space sea, Vietnam is inhabited by many marine organisms in general and in particular marine mammals. Currently in Vietnam there is no complete statistics about the composition, quantity, and distribution of marine mammals in the sea areas of Vietnam. However, annual the phenomenon of marine mammals (whales, dolphins, dugong) occur frequently in the waters, some aground, dying washed ashore has been recorded. Besides, Vietnam is a country with worship of Ong fish (whales, dolphins) so as skeletal specimens of this species are stored in the museum, the temples of fishermen construction. This report aims to bring together and provide initial information on cetaceans in the waters of Vietnam in recent years in response to a question about the species composition, number and distribution of marine mammals in Vietnam.

#### Materials and methods research:

*Research documents:* Gathering all of the research on marine mammals in Vietnam from scientists who studied in past time. The sources of information related to marine mammals in the mass media, the agency give the authors, the samples were stored marine mammals, on display at the museum majors, the church of Ong fish in the coastal areas of Vietnam. Also some questionnaire on the status of marine mammals are collected from local people in: Bach Long Vi, Con Co, Phu Quy, Phu Quoc and Tho Chu islands.

*Research Methodology:* Method aggregate and data analysis of previous studies on marine mammals in Vietnam. Interview method based on the standard form designed for different target groups such as fishermen, staff management, conservation officers. Fieldwork methodology, approach the samples are stored in specialized museums, the mausoleum worship of Ong fish at the local. The samples identification based on morphological methods of different authors.

#### Results and Discussion:

The study of marine mammals in Vietnam has been done before: Smith et al., 1995; Dao Tan Ho., 1999; Andersen and Kinze., 2000; Nicholas J. Cox., 2002; Smith et al., 2003; Chiou-Ju-Yao et al., 2011; Pham Van Chien et al., 2013.

In general, these studies are referring to components marine mammal in research areas of Vietnam. Method is mainly used methods based on morphological characteristics as well as the skeleton body.

These studies were mostly done in the 2000s by foreign scientists. Besides, there is also a rare research methods used by survey boats to observe and describe the natural marine mammals in the Gulf of Tonkin of Vietnam. These studies showed that in Vietnam there are about 20 species of marine mammals occur in different areas. Some widespread species such as finless dolphin, dolphin spotting as well as some tropical species found only in certain waters, such as sperm whales and gray whales.

On the subject dugong in Vietnam now only recognized their presence in sea areas of Phu Quoc and Con Dao. However, in recent years to catch them in the wild is rare.

#### Conclusion:

In the sea areas of Vietnam is that about 20 marine mammal distribution, some species have a wide distribution as finless dolphins, spotted dolphins tropical. Some species are only found in certain regions, such as gray whales, sperm whales.

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**Family Ziphiidae in Asia: Two Case Studies on Population Genetics of the Baird's Beaked Whale *Berardius bairdii* and Stejneger's Beaked Whale *Mesoplodon stejnegeri* around Japan**

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The family Ziphiidae, so-called beaked whales, is the second largest group of the Odontoceti. The family consists of currently recognized 21 species in six genera. Most species of the beaked whales are rarely seen at sea and the biological information is mainly from stranded animals. Molecular genetic analyses are powerful tool not only for species identification but to clarify population structure, and phylogenetic relationships. To understand the intraspecific population structure will be the basic part of the life history studies.

In Japan, we have collected 69 of *B. bairdii* and 97 of *M. stejnegeri*. For *B. bairdii*, the fishery people and crew of watching boat suggested two different external morphologies in the Sea of Okhotsk area. Molecular studies, however were not made until recently. We started collecting specimens around Japan cooperating with universities, stranding networks, and research institutions. For population genetics, nuclear DNA  $\alpha$ -2 actin gene first intron (ACTA2I) and the complete mitochondrial (mt) DNA control region (CR) were used to estimate genetic differences. As a result, the possibility of a cryptic species was shown in genus *Berardius* (Kitamura *et al.* 2013). For *M. stejnegeri*, general biology, life history and so on have been studied in the eastern and western North Pacific (Walker & Hanson 1999), but no substantial studies on the population structure had been made. Using two DNA markers mentioned above, we estimated genetic diversity and conformed to Hardy-Weinberg equilibrium, *M. stejnegeri* around Japan suggested to be one panmixic structure.

Our findings must be confirmed with a larger scale study using more specimens from different regions of the distribution range. In this context, international cooperation is essential to collect whale specimens for finer molecular genetic analyses of population structure. Also collecting stranded specimens, bycatch information, external morphometry, the materials necessary to DNA analyses, such as skeleton, muscle and skin will be helpful for compiling the basic information to understand them biologically.

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**Chronological, Spatial and Age Distribution of Finless Porpoises (*Neophocaena phocaenoides*) in Taiwanese Waters Based on Stranding, Bycaught and Landbased Sighting Surveys**

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**Keywords:** finless porpoise, stranding, bycaught, landbased sighting, Taiwan

**Introduction:** Finless porpoises are small-sized toothed-whales inhabiting both in marine and freshwater areas. Their distribution is limited to Asian shallow coastal areas, river mouths, around some islands and the Yangtze River basin in China. They are one of the most commonly seen cetaceans in Taiwan Strait. In the Redlist issued by IUCN in 2011, finless porpoises were ranked as vulnerable. Consequently, appropriate conservational management will be an important task in the future.

**Materials & Methods:** In order to understand the distribution pattern of the finless porpoises in Taiwan waters, we collected the specimens of finless porpoises which had died due to stranding or fishing incident during the years of 1994 - 20013. Geological site and date were recorded for each stranding and fishery interaction. Body length, sex, and age were investigated for each specimen. Starting from the year 2005, we conducted a three-year landbased sighting survey to monitor finless porpoises' occurrences near the coast of Matzu Islets in western Taiwan Strait. Chronological and spatial distribution were analyzed by using ArcGIS. Descriptive statistics were carried out by using SYSTAT.

**Results:** Our survey results indicate that from October to the following April are the months when finless porpoises most frequently die due to stranding or fishing incident. The majority of the carcasses of the porpoises, with the body length ranging from 71 to 170 centimeters, were found in the western coast of Taiwan Island and in the northward shores of islets scattered in Taiwan Strait. Age structure was categorized into 5 classes as neonate (age 0), calf (age 1-1.5), young (age 2-4), adult (age 5-10) and old (age more than 10). Class young was most frequently found (30%) among the five age classes, followed by calf (26%), adult (20%), neonate (14%) and old (10%). For the landbased surveys, sighting Index (hereafter SI, sighting/hour) and Total Recoded Porpoise Presence (hereafter TRPP, total porpoise presence /hour) were significantly higher in the period of north-east monsoon season than in the period of south-west monsoon. In addition, SI and TRPP for summer were lower than those of spring, fall and winter.

**Discussion:** Both the records of the dead samples and the sighting of the live finless porpoises suggest that from autumn to the following spring are the seasons when finless porpoises constantly appear. In addition, 70% of the porpoises died before the age of 5 which is supposed to attain sex matured for the porpoise. The higher mortality of sub-adults imply that proper conservation management shall be proceeded. Hence we propose that the conservation of finless porpoises in Taiwanese waters should be adjusted based on the seasonal differences. We also suggest that some measures on fishery control should be taken. Furthermore, continuous monitoring of the activities of finless porpoises is essential.

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**First Record of Ginkgo-toothed Beaked Whale (*Mesoplodon ginkgodens*) stranded in Korea**

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**Introduction:** Two stranded dead whales (one adult and one calf), which are suspected to be Genus *Mesoplodon*, were found near the coast of Jeon-heul dong, Obong-ri, Udo-myeon, Jeju Island, South Korea on 5th May, 2013. As there are few cases of stranded *Mesoplodon* in Korea, these two bodies were dissected and observed for some taxonomic and veterinary information.

**Materials & Method:** Genomic DNA was extracted from the muscles. About 500 bp of the 5' half of mitochondrial (mt) DNA control region was amplified and sequenced by the pair of primers (Dalebout *et al.*, 2004). For species identification, the BLAST search (NCBI: <http://blast.ncbi.nlm.nih.gov/Blast.cgi>) was used. After the observation of the outer appearance, the dissection was performed from both the anatomical and pathological point of view. Skeletal specimens were prepared after the dissection and their morphological characteristics were compared with the holotype specimen of Ginkgo-toothed beaked whale stored in the National Museum of Nature and Science, Japan.



**Results:** The adult specimen was found to be female with a total body length of 456.5cm, and many scars on the surface, probably made by cookie cutter shark attacks, were observed. The calf body was also found to be female and its body length was 199.0cm. With the BLAST search, these two whales were identified as Ginkgo-toothed beaked whale. Morphological comparison of the adult skull specimen with the holotype specimen also confirmed the species identification.

**Discussion:** This is the first record of stranded Ginkgo-toothed beaked whale in Korea. Although further genetic analysis is needed to confirm the precise relationship between the two whales, these were speculated to be a dam and a calf according to the evidence including stranded situation, body size, developed mammary glands and stomach contents. This record might provide valuable information including the habitat and gestation of this species.

**Keywords:** Ginkgo-toothed beaked whale, *Mesoplodon ginkgodens*, stranded beaked whale in Korea

## BEAR

### Bear Bile Farming and Its Impact on Welfare and Conservation of Bears in Vietnam

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#### Abstract

Vietnam has two species of bear, the Asiatic Black Bear *Ursus thibetanus*, also known as Moon Bear, and the Malayan Sun Bear *Helarctos malayanus*. Both species of bear are legally protected under Vietnamese law against hunting, trapping and keeping of bears. The trading of any bear products including bear bile is also prohibited. Furthermore, these species are protected under CITES (the Convention on International Trade in Endangered Species) in which Vietnam is one of 180 countries that are signatory to this treaty. However, bear bile farming over the past decades has driven both species to a critically endangered level. Estimates in 1999 indicated approximately 400 bears kept for bile farming in Vietnam, the ongoing demand for bear bile has pushed this figure to over 4000 in 2005. The majority, if not all bears, kept for bile extraction are wild caught bears either from Vietnam or from the neighbouring countries. Bear bile farming has heavily depleted wild bear populations in Vietnam and are believed to be locally extinct most provinces. Animals Asia in collaboration with Vietnam's Ministry of Agriculture & Rural Development has established Vietnam Bear Rescue Centre in Tam Dao National Park in 2008 with the aim of ending bear bile farming in Vietnam and rehabilitate bears that are rescued from bear farms and illegal trade.

### Management of Rescued Bears from Bear Bile Farms at Animals Asia Sanctuary in Vietnam

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Across Asia, up to 20,000 bears are cruelly farmed for their bile despite the herbal and synthetic alternatives that exist. Animals Asia is devoted to end the practice of bear bile farming and we have rescued over 400 bears in China and Vietnam since 1994. In Vietnam the practice of bear bile farming is illegal but approximately 2,400 bears remain confined on farms throughout the country where they are continuously extracted of their bile. In Tam Dao we have established a rescue centre which can accommodate up to 200 bears once completed. Of the 120 bears rescued (*Ursus thibetanus* and *Ursus malayanus*), 112 bears are still alive. Rescued bears vary in age from 2 month old cubs to bears who have been kept on farms for 15 years.

Bears on bile farms are kept in small cages in a non stimulating environment where they endure tremendous physical and psychological suffering. When they arrive at our sanctuaries they have gone through a lot of trauma and it takes time to adjust to a new life. Among the groups of bears we manage, a large number are physically challenged – blind, missing limbs, claws and teeth.

Considering that the bears we rescue tend to be solitary, they have a traumatic past and they vary in age and disabilities, we face many challenges to successfully house them in social groups. After their rescue, they are moved into quarantine where they receive a balanced diet and enrichment for the first time. This will aid in their rehabilitation after years of severe neglect. They will then move into a den in a bear house with outdoor enclosures where they will be able to socialise with neighbouring bears. Cautious steps are taken throughout the process of integrating the bears into social groups.

Consideration is given to enclosure design to provide the bears with sufficient areas to forage, rest and play. An extensive management programme plays an integral role in the recovery of traumatized bears. Ongoing monitoring of their behaviour and health is accommodated throughout to ensure the bears optimum welfare.

**Keywords:** bear bile farming, management programme, welfare

### Operant Conditioning as an Animal Management Tool

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At Animals Asia Vietnam Bear rescue centre we currently house and manage a population of 112 bears: 8 Malayan sunbears *Helarctos malayanus* and 105 Asiatic black bears *Ursus thibetanus*. Managing such a large population of socially housed bears can present its own issues, such as separating bears, recalling between enclosures and dens and weighing and loading bears for health checks. Positive Reinforcement Training provides the means to mitigate social problems, aid in introductions, reduce abnormal behavior, enhance enrichment programs, increase the safety of attending personnel and aid husbandry & veterinary procedures.

**Keywords:** Operant Conditioning, Animal Management, Positive Reinforcement

### The Role of the Veterinary Nurse in Wildlife Medicine and Conservation

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#### Abstract

Although the role of the veterinary nurse has developed significantly in recent years veterinary nurse positions in zoos and wildlife centres are still few and far between. Veterinary nurses undergo intensive training and possess a wide range of skills which can be utilised in the field of wildlife medicine and conservation. In addition to looking after animal patients and supporting the veterinary team, veterinary nurses can assist veterinary surgeons with laboratory and clinical procedures including taking blood and anaesthesia. They can also distribute and give medications/treatments as directed by a veterinary surgeon, assist in the capture and handling of animals, help to keep medical records, assist with post-mortem examinations and keep the veterinary facilities clean and well stocked. Veterinary nurses are an asset to any organisation specialising in wildlife medicine and conservation and should be considered an essential part of the team.

### Common Health Problems of Previously Bile-farmed Asiatic Black Bears (*Ursus thibetanus*) in China

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#### Abstract

Animals Asia's award-winning sanctuaries in Vietnam and China manage the largest population of previously bile-farmed Asiatic black bears (*Ursus thibetanus*) in the world. Since 2000, Animals Asia's Chengdu Bear Rescue Centre has rescued 285 Asiatic black bears and Eurasian brown bears (*Ursus arctos*) from bile farms in China, where bears experience serious health and welfare issues. Aside from extensive problems directly associated with the bile extraction process, rescued bears exhibit and develop numerous additional health problems throughout their lives. Common health problems in

resident bears include dental disease, mobility/lameness, cardiac disease, along with weight management and ingrained stereotypic behaviors developed on bile farms. Dental extractions have been performed in 215 of 243 (88.5%) bears due to canine fractures and/or severe wear secondary to chronic stereotypic bar-biting behavior and/or intentional cutting of canine teeth by bile farmers. Of the 130 bears currently living at CBRC, clinical gait abnormalities are exhibited by over 57 (43%) bears and 49 (38%) are on long-term medications to manage degenerative joint disease. 25 (19%) of 130 resident bears are currently being medically managed for cardiac diseases comprising aortic aneurysms, dilated cardiomyopathies, and/or suspect hypertension based on a combination of retinal pathology, chest radiographs and/or echocardiographic findings. To the authors' knowledge, aortic aneurysms and hypertension have not been previously reported in bears. Weight management is an additional challenge as rescued bears are housed in mixed-sex social groups. Feeding regimes are complicated by the highly seasonal foraging behavior of bears, further compounding efforts to maintain lean body conditions to alleviate mobility and/or cardiac issues. Additionally, bears develop stereotypic behaviors on bile farms due to chronic pain, stress and deprivation which become ingrained, difficult to break, and can exacerbate concurrent health issues such as degenerative joint disease. Of 155 deceased bears, 62 (40%) died of cancer with liver cancer comprising 29% (n=46) of all deaths. The second leading cause of death is spinal paralysis/paresis or deteriorating mobility, accounting for 25 (29%) deaths, followed by cardiac disease, 17 (11%). To further investigate the presence and degree of some of these main health issues affecting previously bile-farmed bears, examinations of free-ranging Asiatic black bears have been conducted in Japan to obtain baseline comparative data. Preliminary findings will be presented.

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#### Retinal Pathologies of Previously Bile-farmed Asiatic Black Bears (*Ursus thibetanus*) in Vietnam

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#### Abstract

The cruel practice of farming bears for the extraction of bile is illegal in Vietnam, but large numbers remain on farms throughout the country. The extraction of bile from these bears, as well as their conditions - malnourished in small cages with metal bar floors - has grave consequences for their welfare and physical state. Since 2008, Animals Asia's Vietnam Bear Rescue Centre has rescued 120 bears, of which 112 are still living at the centre: 8 Malayan sunbears *Helarctos malayanus* and 105 Asiatic black bears *Ursus thibetanus*. Common health problems detected in rescued bears include those directly related to the extraction process (biliary and hepatic pathologies), as well as those related to husbandry: dental fractures/severe wear due to stereotypic bar-biting, mobility issues due to their confinement in small cages or as a result of trapping in the forest, and stress-related disorders. The Vietnam Bear Rescue Centre has a well-equipped veterinary hospital, and bears are given extensive health checks for an early diagnosis of potential health issues, using e.g., ultrasound and indirect ophthalmoscopy. A comprehensive eye exam, including a thorough retinal exam under dilation, can detect early signs of numerous diseases that affect not only the eyes and sight, but also overall health. Some of the retinal pathologies seen in a number of our bears using binocular ophthalmoscopy include retinal haemorrhages and beading of retinal vessels. Although the list of differential causes for these symptoms is long, many could be discarded following further diagnostics or therapeutic trials, leaving a presumptive diagnosis of hypertension for most of these bears. Using ophthalmoscopy, we are therefore able to preventively treat our bears for hypertension before detrimental effects (e.g., aortic aneurysms) are seen. A variety of retinal lesions seen in the bears, as well as the further diagnostic and treatment protocols trialed to test various hypotheses (such as vasculitis) will be discussed, as well as cheaper alternatives to traditional binocular ophthalmoscopy, e.g., using standard DSLR or smartphone cameras.

#### Heat Stroke Case in Male Sunbear in BOS Foundation Samboja Lestari, East Borneo

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#### Abstract

Heat stroke is a syndrome caused by an extremely high temperature and humidity, or strenuous physical exercise. The body denied to compensate the high temperature which lead to body malfunction (Bruchim et al. 2009). Bruno was a sun bear living in SB Sanctuary BOSF SL, East Borneo, it was an adult male sunbear (13 years old) and 45 kg in BW. Bruno stayed in social enclosure with others seven adult male sunbear. On December 12, 2010, at 13.00 WITA Bruno was found in unconscious condition after fought in the male social enclosure. The microclimate in that day was hot with 31°C of temperature, and 90% of humidity in the air. From the general check up, Bruno's condition showed many biting wounds, dyspnoea with short and deep breath. The gingiva was cyanosis. Treatment : NaCl 0.9 % was given by soaked the infus set in the cold water to make NaCl 0.9 % fluids cold and we compressed with the ice over the body surface (Kreeger, Arnemo, Raath 2002). Treatment was given for 30 minutes before Bruno finally death at 13.30 WITA. From necropsy, the abdomen cavity feel hot and there was abdomen fluid about 50 ml with 38.3 °C of temperature. The heart looked contracted. From macroscopic diagnose result, Bruno had organ malfunction caused by hypertemia.

**Keywords :** Sun Bear, Strong Exercise, Fought, Heat Stroke, Organ Malfunction

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#### Ketamine and Xylazine as Chemical Restrain Agent in Borneo Sunbear (*Helarctos malayanus euryspilus*) at Kalaweit Gibbon Conservation Center and Sanctuary

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**Introduction:** The information about anaesthesia in Sun Bear is considered to be lacking compared to other species of bears (Caulkett and Cattet, 2002; Caulkett, 2007). The combination of Ketamine and Xylazine, although formerly were the drugs choice for bear immobilization are now considered to best be avoided if necessary as it is considered to be unreliable especially for its known risk of sudden recoveries (Caulkett and Cattet, 2002). Other characteristic known including risks to the animal include convulsions, and hyperthermia. Xylazine can be antagonized with yohimbine, but since a high dose of ketamine is required, adverse effects of the ketamine (rigidity, convulsions, hyperthermia) are unmasked (Caulkett and Cattet, 2002).

**Material & Methods:** Five sun bears were chemically restrained with the total of fifteen times using the combination of ketamine and xylazine during 2013-2014. This paper was written based mainly on those seventeen cases with supportive data from every cases of chemical restrain since 2008 when the first sun bear came to the sanctuary. The dose used was 3 mg/kg ketamine combined with 1 mg/kg xylazine. Both chemicals were mixed in one syringe and either injected using a compressed syringe dart with a blow pipe or injected by regular syringe if possible. The actual body weights were not necessarily possible to be measured so the calculation of the dose was based on approximate estimation. The pulse and respiration were counted whenever possible so were the measurement of body temperature. Any characters observed during anesthetized period were noted. The antagonist of xylazine, yohimbine were applied whenever considered to be needed.

**Results:** Induction achieved in less than 5 minutes whenever the drugs were successfully delivered. In a case of failed induction, the second doses (full dose or ¾ dose) were delivered within the range of 8-10 minutes. Soon after induction two individual were having a deep long breathing which lasted less than a minute after which the breathing were just normal. Both individual were immobilized twice but only experienced the said kind of breathing one time. Two individual experienced vomiting at induction time. Two bears experience heavy lower abdominal contraction at the end of induction. Despite that Ketamine are characterized by an opened eye during anaesthesia, one bear closed its eyes during immobilizations. Nystagmus was once

noted. Sudden recovery was once encountered. A slightly tremor or ataxia at the extremities were sometimes occurred. Breathing ranges from 16-28 times per minute. Pulse counted 72-84 times per minute. Body temperature ranges from 37.5-39.0°C.

**Discussion:** Being alpha-2 adrenergic agonist vomiting is one of the expected side effects of using xylazine and therefore it is recommended to withhold the food for 6-12 hours to reduce the risk of emesis. According to one study on 22 immobilizations using another alpha-2 adrenergic agonist, medetomidine, on sun bear by Onuma (2003) 16 of them were vomiting despite being adequately fasted for 17-20 hours before immobilization. Furthermore, being monogastric, bears can be prone to vomiting or regurgitation during induction of anaesthesia. Therefore it is best to avoid anaesthetizing bears that have recently eaten (Caulkett and Cattet, 2002). But due to condition on the field it wasn't always possible to do so as sometimes cases where chemical restraint were needed were happening after the food was given to the animals. Due to the routine in Kalaweit, mostly bears were immobilized about 2.5-3 hours after they were being feed. At this timing there were never any problem related to vomiting except two times where two bears experiencing a vomiting at induction. The one individual was received very high dose due to multiple failure of induction that when it was finally immobilized it experienced heavy abdomen contraction that eventually resulting on vomiting at the end of induction time. The other one was observed to be eating the remaining food from early feeding just before the immobilization took place. The bolus appears to be fresh and not likely being digested just yet. However on another case during a rescue, one bear was distracted by food so that it possible for it to be injected with drugs, was not experiencing any problem of vomiting. The same bear was then immobilized one month later and was again distracted by food and no vomiting occurred, despite it was injected by double dose due to failure of the first induction.

It was appeared that vomiting was mainly influenced by two things: heavy contraction on lower abdomen and the position of the animal during induction time. This was based on observation made on three individuals that experienced those characteristic. The first bear was fell on ventral classic "dog sitting position" and was having a very heavy contraction on its lower abdomen until finally it was vomiting, but the still partially conscious bear swallowed the bolus back. The second bear was falling at the same position and having the same heavy abdominal contraction followed by salivation, but before any vomiting occurred we turned the bear position into lateral position. The last bear showed no marked contraction on the abdomen but the position of the head was hanging low as it about to unconscious.

Salivation sometimes occurred at induction time but not always. Nystagmus was once seen at the end of induction time in one individual. At the end of induction two individuals experiencing a deep breathing with a long interval that lasted for  $\leq 2$  minutes after which the breathing was normal throughout the anaesthesia period. Sudden Recovery was once encountered. The sun bear was anaesthetized for about 30 minutes and was having its wound on its palm stitched when it suddenly woke up and slowly walked away tottering. Deep breathing was observed before it woke up and the next time the same bear was being anaesthetized the same character of breathing was again observed before it woke up.

Another characters noted was ataxia. One bear was having some series of minor ataxia on its lower extremities after given the riversine which might be due to a complete reversed of xylazine by riversine leaving the animal under the pure effect of ketamine. Another bear receiving a relatively high dose was experiencing some series of minor ataxia on its upper extremities throughout the anaesthesia period. Twitching on the muzzle and mouth were also often noted.

Because Xylazine depresses thermoregulatory mechanisms with either hypothermia or hyperthermia may occur depending upon the ambient temperature (Lukasik, 1999), at Kalaweit we carefully not perform immobilization on extreme temperature unless it was urgent (which has never happened). At the relatively normal ambient temperature (according to our location, Borneo) the body temperature ranges from 37.5° to 39.0°C. Whenever possible the body temperature was measured three times at the onset, at the middle, and at the end of immobilisation before the reversine was given. The difference degree of which were never really significant ranging from 38.9° to 39.0° to 38.8°C and 37.8° to 37.5° to 38.1°C.

**Keywords:** Sun Bear, Ketamine-Xylazine

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#### Sunbear (*Helarctos malayanus*) Amputation at Kalaweit Supayang Gibbon Convention Center and Sanctuary

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**Introduction:** Sun bear (*Helarctos malayanus*) is the smallest in size and least known bear species. This is the only tropical bear species inhabiting lowland tropical rain forest throughout much of southeast asia (Servheen, 1999). The sun bear is listed under Appendix I in CITES and vulnerable in IUCN. Sun bear habitat was reduced and damage due to human encroachment on Indonesia forest. Human-wildlife conflict is increased since then (Fredriksson, 2005).

**Materials & Methods:** A wild 3 years old female sun bear was delivered to Kalaweit Supayang Gibbon Conservation Center and Sanctuary by the Natural Resources Conservation Office (*Balai Konservasi Sumber Daya Alam/BKSDA*) of Pasaman, West Sumatra with an open wound in sinister front limb, caused by amputation which was done 5 days earlier. Amputation was done as a result of snare trap injuries. This bear was very alert but had good appetite. One day after the arrival, this bear was sedated with 3 mg/kg ketamine combined with 1 ml/kg xylazine. Anaesthesia was administered with blowpipe. After fully sedated, the bear was examined. 1/3 distal humerus was seen from an open wound in sinister front limb. There was also a necrotic area in the muscle surrounding that humerus. The necrosis suggested that wound was sustained several days prior. Wound was cleaned thoroughly and thereafter, the visible 1/3 distal sinister humerus was amputated.

**Results:** The bear was recovered well after surgery and is placed in a concrete floor cage. She was given antibiotic, anti inflammatory and painkiller. The surgical area was not bandage. There was a discharge and the surgical site was half opened 2 days after the surgery, probably because the bear can lick the surgical site. One and a half month after the surgery, the wound was totally healed and this bear had adapted well to amputation although it will preclude her return to the wild.

**Discussion:** This bear suffered significant trauma inflicted by a trap. Unfortunately, this represent the current threats to Indonesian wildlife in general. The lack of balanced land use planning, with large forest area being cleared for plantation development or logging, plays a big role in undermining sun bear protection in Indonesia. Law enforcement regarding wildlife crimes is lack in most areas. These factors, together with the occurrence of serious forest fires each dry season, affect much of remaining sun bear habitat (Tumbelaka and Fredriksson, 2006). In the majority of cases, therefore, effective conservation will demand some means of peaceful coexistence of people and wildlife outside protected area (Woodroffe, 2005).

**Keywords:** sun bear, humerus amputation

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## POSTER SESSION

### ANATOMY AND PHYSIOLOGY/ WILDLIFE MANAGEMENT AND CONSERVATION

#### A Study of the Hematological and Serum Biochemistry Values of Tigers (*Panthera tigris*) in Captivity in a Korean Zoo

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**Abstract:** Blood samples of 38 tigers (30 Bengal tigers and 8 Siberian tigers) were tested to standardize hematological and serum biochemistry values. Differences of hematological and serum biochemistry values among subspecies were verified. The results of this study would be useful for clinical evaluation of each subspecies.

**Key words:** Bengal tiger, Siberian tiger, hematological values, biochemistry values, blood analysis, international standard references

**Introduction:** This study compares the hematological and serum biochemistry values of tigers currently in captivity in Korean Zoo with international standard references in order to establish a standard for captive tigers in Korea. It also compares the hematological values of Bengal tiger and Siberian tiger in order to establish previously unknown standard references for these tigers of different habitats.

**Materials and Methods:** The objects of this study were 38 tigers (30 Bengal tigers and 8 Siberian tigers) in captivity in Korean zoos from 2007 to 2013. The tigers have no apparent health issues, and had their blood collected under anesthesia. Data from blood analysis was compared for statistical significance with international reference values (ISIS), subspecies, sex and age. The results were compared with significance level of .05(p<.05).

**Results:** Eight of the experimental data in comparison to international reference values (ISIS) were of significant statistical difference. Comparison with subspecies yielded 10 and 8 items of statistically significant differences respectively for Bengal and Siberian tigers. Comparison within subspecies revealed 12 items of statistically significant differences. Comparison of sex and sexual maturity revealed 4 and 9 areas of statistically significant differences.

The study revealed 4-12 items of statistically significant differences.

**Discussion:** Currently, International reference values (ISIS) offers hematological and serum biochemistry values of species which is applied to subspecies as well. In this study, blood analysis of Bengal and Siberian tigers showed that subspecies of *Panthera tigris* have significantly different serum analysis values. From this result, standardization of blood analysis data among subspecies would be used for detailed diagnosis.

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#### Morphology of the Korean Water Deer, *Hydropotes intermis argyropus*, Placenta

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**Introduction:** Generally, the chorioallantoic placenta in ruminants including deer is cotyledonary, non-deciduata and epitheliochorial (or synepitheliochorial) in type. However, there are many differences in morphological aspects such as the number, size and shape of placenta, and besides the distribution and number of binucleated cells (BNC) among ruminant placentas. There is no report about the Korean water deer placenta. Thus, we addressed some morphological aspects of this deer placenta.

**Materials & Methods:** Two nonpregnant uteri and a total of 9 pregnant uteri were used in this study. All of uteri were collected from road-killed animals and stored in Seoul National University. Pregnant uteri had fetuses from 92.8 to 194.5mm in CRL, respectively. After studying the number, size, volume and shape of placentomes, tissues were fixed by 10% buffered formalin and embedded in paraffin. 4µm thick sections were cut and stained by H-E, PAS, Azan stain. Then, the distribution and number of BNC were analyzed.

**Results:** The number of fetuses was 1 to 4 (usually 3), while the number of placentomes was 5 to 9 (usually 6), indicating that each fetus occupied 2 to 3 placentomes and that its number was much less than those in cows and ewes. Both the total volume of placentomes and the volume of placenta per fetus was increased 8-9 fold, when comparing CRL 92.8mm placenta to CRL 194.5mm placenta. Water deer placentomes had a convex surface with a more distinct stalk than cows. Chorioallantoic villi were covered by a simple layer of epithelium composed of two cell types, columnar cells and BNC. Since BNC were well differentiated in all samples tested, when BNC first appeared remains to be an experimental question. Cell density of BNC in placentomes was increased 2-3 fold with gestation proceeded. Migration of BNC to the cryptal uterine epithelium was observed even in 194.5mm placenta, suggesting that its migration can continue throughout pregnancy, unlike that of cows.

**Conclusion:** This study clearly showed that the Korean water deer placenta had characteristics in some morphological aspects.

**Keywords:** binucleated cell, Korean water deer, placenta

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#### Changes in Milk Protein Concentration during Lactation in Captive Malayan Tapirs (*Tapirus indicus*)

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**Introduction:** Milk provides optimum nutrition during the natural lactating period in mammals and is an important factor for successful infant growth. Milk protein is the most important nutritional component in milk and its concentration changes significantly during lactation in many mammals. Understanding how milk protein concentration varies is essential for captive breeding programs of endangered species, because it provides information

about weaning and the timing of immune transition in infants. However, the concentration of milk protein in milk from the Malayan tapir (*Tapirus indicus*) and how it changes during lactation is unknown. Furthermore, due to the low lactation yield from this species, it is difficult to measure milk protein levels using common procedures such as the Kjeldahl, dye-binding, and Lowry methods, which require a high-volume milk sample.

This study aimed to see how milk protein concentration be measured in small-volume milk samples from the Malayan tapir using the dry chemistry method and to research milk protein concentration during lactation.

**Materials & Methods:** Two Malayan tapirs were used in this study (Internal Studbook No.060 and No.061). Animals were maintained at the Preservation and Research Center, Yokohama, Japan. No.060 gave birth on September 3, 2008. No.061 gave birth on August 31, 2006 and October 12, 2008. Milk samples (0.5–1.0 mL) were collected twice a week or once a month and were frozen at -25°C soon after collection until analysis. Milk protein (Total Protein: TP) was measured using FUJI DRI-CHEM 3030 (FUJIFILM Co., Ltd.) as dry chemistry. Accuracy of the assay was demonstrated by a dilution linearity test.

**Results:** Milk protein of the Malayan tapir could be measured using a 10 µL sample by the dry chemistry method. The intra-assay coefficient of variation was  $1.3 \pm 0.54\%$  (mean  $\pm$  SD). A linear relationship was identified by calculating a regression line by the method of least squares. The coefficient of determination was within the limits of 0.998–0.999 ( $P < 0.001$ ). Milk TP concentration was found to change during the lactation period using this method. Milk samples were collected from No.061 on the day of delivery. The colostrum TP concentration was measured and found to be 9.2 g/dL in 2006 and 10.3 g/dL in 2008, respectively. Milk TP levels were highest during the first year after delivery in milk from both individuals. The TP values in milk decreased significantly within the first 24 h and were 4.1 g/dL and 3.6 g/dL, in 2006 and 2008, respectively. The amount of TP in the milk from No.060 varied from 2.5 to 5.4 g/dL ( $3.8 \pm 0.52$  g/dL) during days 5–236 after delivery. Following delivery by No.061 in 2006, the milk TP levels were found to vary from 2.1 to 4.6 g/dL ( $3.8 \pm 0.56$  g/dL) between days 1 and 238. There were no significant changes in milk TP concentration of both individuals, No. 060 and No. 061, during the above mentioned period of each ( $P > 0.05$ ). After about 240 days, a slow increase in milk TP concentration was observed in both individuals. The milk TP concentration from No.060 was 6.8 g/dL at 306 days after delivery and for No.061 (2006) was 8.0 g/dL at 328 days after delivery. This increase in milk TP concentration was the highest after that occurring in colostrum.

**Discussion:** This study indicates that the dry chemistry method can be used to measure milk TP concentrations in milk from the Malayan tapir. It has been reported that the high milk TP concentration in the colostrum is due to the presence of immunoglobulins. The timing of immunoglobulin secretion in milk varies between species, and was reported to occur at 24 h in cattle (*Bos Taurus*) and goats (*Capra hircus*), 24–36 h in pigs (*Sus scrofa domestica*) and llamas (*Lama glama*). This study supports that milk from the malayan tapir which was collected within 24 hours contains the secretory immunoglobulin. Therefore, it is critical to check if newborn Malayan tapir infants can suckle within the first 24 h after birth in captivity. As milk TP concentration is significantly elevated towards the end of the lactation period in farm animals, this change can be used as an index to determine the end of lactation. As a similar increase was observed here in Malayan tapirs, it is assumed that lactation would end around 240 days after delivery. In captivity, therefore, it is suggested that the infant should be kept together with the mother for at least 8 months after delivery.

**Keywords:** Malayan tapirs, lactation, milk protein

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#### Management Evaluation of Zoo Elephant in Korea

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#### Introduction:

Elephants are the largest land animals in the world, and one of the representative animals of the zoo, and popular to visitors, especially children. Elephant is long-lived and highly intelligent animal with a complex social behaviors such as greeting ceremonies, group defense, vocal communication, social play and so on (BIAZA,2010). There are currently two recognized species of elephants, the Asian elephant and the African elephant. There are several management guidelines in zoo for elephant in the world, including environmental and behavioral enrichment. In spite of 100 years history of elephant at zoo in Korea, there is still no any guideline for management and care, which may enhance the animal's well being.

#### Materials & Methods:

We visited every zoo which had elephant, and listened to manager or keeper for each situation and watched every facility. Questionnaire was made for getting management information of elephant at zoo, based on the AZA Standards for Elephant Management and Care. The items applied here were modified considering domestic situation. Questionnaire was included five items: abiotic environmental factor, biotic environmental factor, health & nutrition, behavior management, the safety & program assessment and it divided into several questions for each item.

#### Results:

There were 8 (6 public and 2 private) zoo in Korea and total number of elephant was 17 (male:female = 9:8), and 1 (2 zoo), 2 (5 zoo) and 5 (1 zoo) animal in each zoo, respectively. However, 3 zoo did not want to share their information and their data did not include. Types of outdoor habitats were made up cement and soil mixed grass but one zoo was covered with entire cement (1 zoo). These animals lived in entire cement showed foot disorder like overgrown cuticle, and split nail. The average space of outdoor was relatively small but its indoor was sized more than 60 sq m, which are larger than recommended. Only elephants in 1 zoo had performed behavioral enrichment program showed relatively normal behavior, others showed abnormal behavior. The size of space may influence this behavior even many factors are involved. There was daily diet recipe in each zoo even its content was similar. Animals could be touched by handler but not trained enough to check health examination such as blood collection, skin and feet care.

#### Discussion:

It is pity that we could not get the information from all of zoo. However, this is the first trial to consider the general condition of zoo elephant in Korea. The older facilities of out- and in-door created more physical and mental problem in elephant at zoo. Most of zoo has no sufficient space, which can influence the behavior. Appropriate space should be available to allow elephants to be separated either through individual stalling or through the use of restraints. It will improve elephant behavioral, social or medical issues. Collecting data of activity, diet, medical, or husbandry of animal at zoo will provide the base of optimal elephant welfare. In addition, elephants must be trained to accept regular medical care, and staff or keeper must be trained to provide that care. Hopefully, more information can be obtained from zoo and it may become basic data improving welfare and management of elephant in Korea

**Keywords:** elephant, zoo, questionnaire, management

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### How Non-invasive Hormone Analysis could be used for Diagnosis and Monitoring Pregnancy in the Sumatran Rhino and Muntjak Deer

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#### Abstract

Non-invasive methods based on urinary and faecal hormone measurements is the most precise of the indirect methods of monitoring female reproductive function. The method has been used to monitor pregnancy in one female Sumatran rhino and three Muntjak deers by measuring faecal progesterin. Faecal hormone analysis was measured using EIA 5-P-3OH and Pg-diol for Sumatran rhino and Muntjak deer, respectively. Pregnancy in the Sumatran rhino was detected by around day 60, when 5-P-3OH value (46.35 µg/g dry faeces) increased above the highest luteal phase values. While in the Muntjak deer, onset of pregnancy was detected around day 155, which Pg-diol reached concentration of 8,08 µg/g faeces. Maximum 5-P-3OH values during pregnancy were ten- to twenty fold (344.2 – 787.07 µg/g dry faeces) higher compare to luteal phase values. Pg-diol in Muntjak could only reach maximum values seven- to eleven fold (18.6 – 29.6 µg/g faeces) higher than luteal values. Gestation length was defined about 475 days and 235 days in the Sumatran rhino and Muntjak deer, respectively. Faecal hormone measurement could not be used for early pregnancy diagnosis, but it could provide profile of faecal progesterin for monitoring pregnancy period.

**Key words:** Non-invasive, faecal progesterin, pregnancy, sumatran rhino, muntjak deer

### Evaluation of Illegal Hunting of Red Deer and Conservation Policy of Department of Environment in Iran

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#### Abstract

Caspian red deer or maral (*Cervus elaphus maral*) is the largest type of deer in Iran. Maral in the past has lived in the north forests of Iran from the Caspian Sea coast, Alborz Mountains Chain and oak forest of Zagros margin from the Azerbaijan up to Fars Province. But the generation of them was completely destroyed in the North West and West of Iran. According to reports about 50 years ago, they have been observed in Arasbaran forests and now a days populations of this species live in protected areas and out of reach of humans. In The present studies data were collected from 2004 to 2014 in the mazandaran state Hyrcanian forest by means of guard of environment and judiciary office of department of environment of mazandaran in this process the all arrested illegal hunting of red deer and the population census, estimation and the correlation of these data was assayed. We provide a first evaluation of how suitable these methods are by comparing the results with population estimates obtained using cohort analysis, and by analyzing the within-season variation in number of seen deer. The data gave us the future of red deer in northern forest of Iran and the results of policy of department of environment in Iran in red deer conservation

**Key word:** illegal hunting, red deer, census, conservation, Iran

### Study on Clinical, Reproductive Indicators and Diseases in Porcupines in Hanoi, Vietnam

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**Abstract:** Study on clinical, reproductive indicators and common diseases in porcupines was carried out on porcupine farms in Hanoi city from July 2012 to August 2013. The study revealed some clinical indicators ( the body temperature, the heart pulse, the respiratory rate) and reproductive indicators (age of porcupines at first mating, the gestation length, the calving interval). In addition, some common diseases were studied including porcupines miscarriage, respiratory disease, gastrointestinal infections. **Key words:** Porcupines, clinical indicators, reproductive indicators, Hanoi city.

**Introduction:** In recent years many species of wild animals have been domesticated. Currently, there are many economic benefits of the breeding porcupines in Vietnam so many farmers are strongly interested in raising these wild animals. However, research on porcupines is limited in Vietnam until now. Studying the biological, physiological, biochemical features and common diseases in porcupines is needed. Therefore, we examined some clinical and reproductive indicators and common diseases in porcupines on the farms in Hanoi suburbs.

**Materials and Methods:** Studied on the porcupines on the farms in Hanoi suburbs from July 2012 to August 2013 and collected data based on selected clinical and reproductive indicators. The porcupines were divided into 3 groups ( porcupines father, porcupines mothers and baby porcupines). The data were processed by the biostatistical methods.

**Results and Discussion:** The study revealed that the body temperature of porcupines in summer was higher than in winter and the baby porcupines had the highest body temperature (in summer was 38,30±0,01°C, in winter 38,24±0,02°C. The heart pulse of porcupine in summer was higher than in winter. The baby had the lowest heart pulse (in summer 42,21±0,14 beats per minute, in winter 42,24±0,11 ). The respiratory rate in summer was higher than in winter. The porcupine mother had the highest respiratory rate in summer 35,56±0,16 breaths per minute, in winter 32,53±0,26 ). Some reproductive indicators of porcupines was also studied. It revealed that age of porcupines at first mating was 9,38 ± 0,65 months. The gestation length was 92,00 ± 1,66 days. The rate of porcupine mothers with 2 babies per litter was 61,11% . The calving interval was 6,18 ± 0,260 months. In addition, some common diseases were studied including porcupines miscarriage, respiratory disease, gastrointestinal infections.

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### Study on Reproductive Indicators in Wild Pigs Raised in Captive Northern Vietnam

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**Abstract:** The objective of this study conducted to investigate the reproductive in pig boar. The results were showed that pig boar starts grow familiar at 150 day of the age, and highest percentage from 171 to 200 day of age (81.67%). The first of breed in the pig boar in Ba Vi farm ranged from 221 to 242 day of age (71.42%) and to give of first birth at 341 to 351 day of age (62,88%). Most of the pig boar had pregnancy long approximately with thin 113 to

115 day. Most of pig boar mother was in heat again after live piglet at later 4 – 6 day. The rate of piglet alive after 2 month was high (86.07%), particularly, the ratio of piglet alive in spring and autumn at 89.41% and 89.47%, respectively and higher in summer and winter (85.19% and 78.46%).

Key words : Piglet, pig boar, first of breed, heat.

**Introduction:** Wild pig livestock is a promising direction of swine industry. Nowadays the demand for meat from wild pigs is increasing. However, reproductive performance of wild pigs is currently still low because research on wild pigs is limited. To develop wild pig industry effectively we examined some reproductive indicators of wild pigs in Northern Vietnam.

**Materials and Methods:** Studied on the wild pigs in Northern Vietnam and collected data based on selected reproductive indicators. Wild pigs were divided into different age groups including suckling piglets, growing pigs, gilts, breeding pigs.

**Results and Discussion:** The study revealed that the age of sexual maturity in male wild pigs in captive raising was from 171 to 200 days with proportion 81.67%. The age of the first mating of wild pigs ranged from 221 to 242 days with the high rate 71.42% and the highest rate (53.90%) was 221 -231 days of age. The age of first calving of gilts was from 341-351 days of age (62.88%). The average pregnancy long of wild pigs was 114,21 days. Most of wild pig mothers were in heat again after 4 – 6 days. The rate of piglet alive after 2 month was high (86.07%), particularly, the ratio of piglet alive in spring and autumn at 89.41% and 89.47%, respectively and higher in summer and winter (85.19% and 78.46%).

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#### Laparoscopic Ovariectomy in the Asian Black Bear (*Ursus thibetanus*) with the use of the SONICISION™ Device

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**Introduction:** Laparoscopic procedures have been accepted as a viable alternative to traditional open procedures in a variety of wildlife species with advantages of reduced postoperative pain and physical stress response. Laparoscopic ovariectomy and salpingectomy have been performed in bears for sterilization or therapeutic purpose. This case report describes the laparoscopic ovariectomy in the Asian black bear using the Sonicision™ device.

**Materials & Methods:** Two female Asian black bears, excluded from species restoration program of Korea National Park Service, were presented for sterilization. Bear A was a 163 kg 13-year-old female, and bear B was a 174 kg 7-year-old female. Bears were positioned dorsal recumbency with the head lower (Trendelenburg position) on a surgical table. A three portal technique was used. A 5-mm cannula was inserted 10 cm caudal to umbilicus (camera port). One 5-mm cannula was inserted 1 cm caudal to the umbilicus (instrument port) and one 10-mm cannula was inserted 8 cm caudal to the camera port (2nd instrument port). An atraumatic grasping forceps and Sonicision™ device were inserted via the instrument ports. The ovarian vasculature, suspensory ligament, and proper ligament were progressively cauterized and transected with the Sonicision™ device. The 10-mm portal site was enlarged to 2 cm of incision length. Through this incision, the ovary was exteriorized from the abdominal cavity. The abdominal musculature, subcutaneous tissue and skin of the portal sites were closed separately.

**Results:** The bears recovered uneventfully, and no peri- or postoperative complications were encountered. The day after surgery, the bears showed good and general behavior of its age and breed. The total surgical times were 113 min in bear A and 49 min in bear B. This is the first report of laparoscopic ovariectomy in the Asian black bear using Sonicision™ device.

**Discussion:** Laparoscopic ovariectomy using the Sonicision™ device is a safe and efficient method of sterilization, and did not require a prolonged surgical time in bears. Further studies are warranted to establish the safe use of laparoscopy in bears.

**Keywords:** Asian black bear, Laparoscopy, Sonicision™, Ovariectomy, *Ursus thibetanus*

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#### Orchiectomy in the Asian Black Bear (*Ursus thibetanus*) with the use of the LIGASURE™ Device

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**Introduction:** Orchiectomy is the best regulation of population in captive wildlife because of surgical simplicity and efficiency. Castration also decreases male aggressiveness, roaming, undesirable urination behavior and androgen-related diseases. This case report describes the use of Ligasure™ device for orchiectomy in the Asian black bear (*Ursus thibetanus*).

**Materials & Methods:** Two male Asian black bears, excluded from species restoration program of Korea National Park Service, were presented for sterilization. Bear A was a 138 kg 7-year-old male, and bear B was a 215 kg 13-year-old male. Surgical procedure of orchiectomy was carried out by closed method using the Ligasure™ device through prescrotal skin incision. Spermatid cord was cauterized with the Ligasure™ device and transected using a blade. Subcutaneous tissues of the incision site were sutured by simple interrupted pattern with absorbable suture material, and the skin incision was closed with tissue glue.

**Results:** The bears behaved normally after recovering from anesthesia. No postoperative swelling or complications were observed. The total surgical times were 26 min in bear A and 24 min in bear B. This is the first case report that describes the use of the Ligasure™ device for orchiectomy in the Asian black bear.

**Discussion:** Orchiectomy using the Ligasure™ device is a feasible method of sterilization, and did not induce major peri- or postoperative complications. This technique seemed to be applicable to other wildlife species.

**Keywords:** Asian black bear, Castration, Ligasure™, Orchiectomy, *Ursus thibetanus*

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### Hematology and Blood Biochemistry Reference Values for Captured Wild Eurasian Eagle Owls (*Bubo bubo*)

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**Abstract:** Hematologic and blood biochemical values were tested with blood samples obtained from 59 wild Eurasian Eagle Owls (*Bubo bubo*). The results of this study will be useful for clinical use and evaluating general health conditions for the rehabilitation of Eurasian Eagle Owls.

**Key Words:** *Bubo bubo*, blood biochemistry, Eurasian Eagle Owls, hematology.

**Introduction:** The Eurasian Eagle Owl (*Bubo bubo*) is a nocturnal bird of prey, largely distributed throughout Europe and Asia. Nevertheless, every year, the number of the sick or wounded individuals brought to wildlife center is increasing. The main objective of this study was to report hematologic and blood biochemistry values for clinical use in Eurasian Eagle Owls. Furthermore, establish reference values for understanding the physiology of the species and for providing proper medical care for wild and captive individuals.

**Materials & Methods:** All Eurasian Eagle Owls (n=59) included in this study were hospitalized in 5 different wildlife rescue centers in Korea. 27 of the birds were considered clinically normal and ready for release. The other 32 of the birds were evaluated to be nonreleasable due to amputations resulting from previous injuries. All of these birds were considered healthy based on normal behavior, appetite, weight and condition for the species (Naidoo et al., 2008). Statistical analysis was performed with the statistical software SPSS and a Kolmogorov-Smirnov test with Lilliefors' correction was used to assess the normality of distribution. Statistical differences were considered significant at  $P < 0.05$ .

**Results:** Total eight hematologic and fore-teen of blood biochemical parameters of wild Eurasian Eagle Owls were examined in this study. Most of hematologic and blood biochemical reference intervals obtained from this study were within or very close to the reference ranges published previously for owls. The parameters that did not mention in those previous reports were compared with reference values of other birds of prey species; and found within those reference ranges.

**Discussion:** We carefully considered the results of this study and determined that they could be added to a table of *B. bubo* reference ranges (Carpenter et al., 2013). Establishment of reference values of hematology and blood biochemistry gives better knowledge for clinical use in wild Eurasian Eagle Owls and understanding of the physiology of those species. However, further studies with larger sample sizes of additional hematologic and biochemical parameters and different environmental factors are required for evaluating pathologic conditions.

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### Rockhopper Penguin (*Eudyptes moseleyi*) Breeding Performance at Underwater World Langkawi, Malaysia

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**Introduction:** Rockhopper penguin, *Eudyptes moseleyi* is northern penguin. Currently, it has been classified as endangered by International Union for Conservation of Nature (IUCN) after very rapid population decreases throughout its range. On December 2003, twenty rockhopper penguins were arrived at Underwater World Langkawi (UWL) and have been in captive for about ten years. The birds started to produce eggs a few years later (2006) at estimated



age is five years after we introduce the suitable nest material. They normally laid two eggs for each breeding season. Both parents were incubating their eggs and then nurse the chick until it fledged. It took about 36 days of incubation (Henry & Sirpenski, 2003) and 10 weeks for a chick to fledge (Croxall & Davis, 1999). Throughout the ten years of breeding season, we are successful to have nine survive chicks.

**Materials & Methods:** Data for breeding and reproduction of rockhopper were collected from 2006 until 2013. All the eggs laid were numbered, labeled and observed their appearance with candling done once a week to monitor their development and to determine the fertility of eggs. This study involved a small sample of breeding population with about six breeding pairs. The breeding performance parameters involved are based on fertility, hatchability and survivability.

**Results:** From six breeding pairs, only three pairs are categorized as productive pairs since they had experienced in raising at least one chick to fledging age. There were 71 eggs recorded from 2006 to 2013. They mostly lay two eggs per season per breeding pair with the first egg (A-egg) are smaller than the second egg (B-egg). The size of A-egg is about  $86.31 \pm 2.14$  g and B-egg is about  $115.54 \pm 3.04$  g. The incubation periods for fertile eggs are within 31 to 39 days with average  $34.56 \pm 1.79$  days. There are two methods of incubation and rearing practiced at UWL which are natural and artificial. From the total number of egg laid, 31 eggs were fertile (43.66%) with hatchability is 54.84% (17 chicks from 31 eggs). Since there are nine survive chicks (out of 31 eggs), the percentage of survivability is 29.03%.

**Discussion:** The rockhopper penguin at UWL usually starts to lay egg from July to November every year. It is almost in the range as reported in the wild (BirdLife International, 2014) and captive rockhopper in North America (Diebold *et al.*, 1999). However, there are a few cases where the females produced only one egg in particular years. The eggs usually lay in 3 to 5 days apart in wild (Croxall & Davis, 1999; Henry & Sirpenski, 2003) but compared to our records, the range is almost similar but there are a few eggs are laid with laying interval between 3 to 8 days.

Percentage of hatchability (54.84%) is lower than recorded for all captive penguin species (76-81%) as reported by Diebold *et al.* (1999). Fertility of the birds is also low especially among inexperienced parents. However, it is said as a common problem in captive population for southern rockhopper. This is probably due to low concentration and low motility of male sperm which contributes to the poor egg fertility in birds (Waldoch *et al.*, 2007). In realizing this factor also may influence the fertility of nonproductive pair, we start to enrich their feed with mussel as a part of their diet. We also just implementing zinc supplement to male but need some time and continuous data collection in determining the effectiveness.

In terms of survivability, only 29.03% were recorded at UWL which is less than reported by Demongin *et al.* (2010) where 0.49 chick recorded per nest when they conducted a study at the Falksland Island. Mostly, the survive chicks are from B-egg. This is due to B-eggs have higher egg yolk masses and yolk androgen levels than A-eggs thus increase the chance of its hatchability and survivability (Poisbleau *et al.*, 2011). Even some A-eggs are recorded fertile; the chick only can survive for a few days. This scenario also reported in wild where hatched chicks from the first eggs almost invariably die during the first few days of brooding (Poisbleau *et al.*, 2008). Since both eggs are viable, they always retain in the wild and captivity.

As a conclusion, there are wide factor that influence the fertility, hatchability and survivability of rockhopper egg at UWL. Since the fertile eggs and chicks usually come from the same productive pairs, breeder maybe the major factor that affects the breeding performance of birds besides egg size, weight, shape and consistency of the contents. Because of no or less comparative study about northern rockhopper penguin in captivity so far, this report maybe can contribute to penguin conservation through captive breeding understanding.

**Keywords:** UWL, rockhopper penguin, breeding, egg, chick, fertility, hatchability, survivability

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#### Histology of Albuminous Glands in Starspotted Dogfish (*Mustelus manazo*) with a Special Reference to Lectin-binding Patterns

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**Introduction:** Although the gestational period of starspotted dogfish is about 10 month, the dogfish has neither yolk sac placenta nor chorioallantoic placenta. The yolk sac is very large in size during first trimester period, but it reduces its size with pregnancy advanced. Finally, the yolk sac disappears during third trimester period. It is known that the albuminous gland is important for nutrient supply. Thus, we analyzed histology of the albuminous gland in starspotted dogfish. Particularly, an attention was paid to lectin-binding patterns in epithelial cells of albuminous glands.

**Materials & Methods:** Albuminous glands were collected from 2 nonpregnant and 4 pregnant starspotted dogfish (approximately 2 mon, 5 mon, 7 mon, and 9 mon of pregnancy). Females were caught in Tokyo Bay. Tissues were fixed by 10% buffered formalin and embedded in paraffin. 4μm thick sections were cut and stained by H-E, PAS, Azan stain and lectin-histochemistry using 21 kinds of lectins.

**Results:** Albuminous glands were located in the junction area between oviduct and uterus, showing heart-shaped. Glandular epithelial cells were divided into six kinds of cell types according to histological characteristics. Type I cells were weakly basophilic; Type II cells showed weakly eosinophilic on the apical site of cells; Type III cells were strongly basophilic; Type IV cells were strongly eosinophilic; Type V cells were moderately eosinophilic; Type VI cells were not reactive due to many vacuoles in the cytoplasm. Type I to VI cells were regularly and lamellarly arranged from inside to outside. Lectin histochemistry revealed that each cell of Type I to VI had specific lectin-binding patterns. Only Type III cells were negative to all of lectins. DBA lectin was positive only in the duct of Type VI. GSL-II, PNA, SJA and PHA-E were negative to all of Type cells. For other lectins, Type IV and V cells showed most strongly reactivity, while Type I and II cells were weak.

**Conclusion:** This study clearly established that there were specific differences in glycosylation of each cell type in albuminous glands, suggesting that each cell has different functions to secrete different nutrient and mucus.

**Keywords:** albuminous gland, lectin-binding pattern, starspotted dogfish

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**Case Study: Mass Death of Frogs (*Ranadybowskii*) caused by Septicemia in Artificial Raising Farm**Yeo-Jin Jeong<sup>1</sup>, Seong-jae Choi<sup>1</sup>, Jong-Taek Kim<sup>1</sup>, Guk-Hyun Suh<sup>2</sup><sup>1</sup>College of Veterinary Medicine, Kangwon National University, Chuncheon 200-701, Korea<sup>2</sup>College of Veterinary Medicine, Chonnam National University, Gwangju 500-757, Korea

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**Abstract:** Frog culture industry, although not well known, has much potential. Generally population density is higher in a farm than in natural habitat. Stressful condition and poor sanitation are likely to occur within an environment with a high population density. Stressful condition and poor sanitation make the population more susceptible to infection and the results more grave.

**Keywords:** frog, bacterial septicemia, helminth, *Cupriavidus metallidurans*, *Pseudoalteromonas* sp.

**Introduction:** Due to severe *Rhabditioidea*-helminth infection and subsequent bacterial septicemia, 50~70% of the total population has been eradicated in a farm in Jeong-sun in Gangwon-do and Chungju in Chungcheongbuk-do from late June, 2012 to September, 2012. Diseased frogs showed ruptured lung, bloody ascites, liver discoloration, myocardium weakness, congested kidney, microcytic anemia and so on. *Enterobacteriaceae*, *Citrobacter* spp., *Cupriavidus metallidurans*, *Acinetobacter* spp. were isolated as major bacterium that had caused septicemia in frogs. Among isolated bacterium, *Cupriavidus metallidurans*, *Ewingella americana*, *Shewanella aquimarina* and *Pseudoalteromonas* sp. have not reported as potential pathogens in frogs before. It is a good example that severe helminth infection in frogs can lead to secondary infection of bacteria.

**Materials and Methods:** Tissue and blood samples were collected immediately after death for various tests. After autopsy, tissue sampling and H&E staining (Hematoxylin and eosin stain) were conducted for 200x microscope observation. In order to identify the bacteria, PCR was carried out with DNA template sample.

**Results:** The main reason for the frogs (*Ranadybowskii*)'s death was due to infection of nematode causing respiratory failure and nutrient insufficiency. Secondary sepsis to infection by a variety of bacteria seems to have led to high mortality.

**Discussion:** Studies indicate that artificial raising farm requires more complicated sanitization control to prevent parasitic infection and secondary bacterial sepsis. If mass death occurs repeatedly, more accurate species analysis should be performed.

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## CLINICAL MEDICINE

**Psittacine Beak and Feather Disease (PBFD), Budgerigar Fledgling Disease (BFD) and Aspergillosis in African Grey Parrot**

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**Introduction:** Avian circoviruses belong to the Circoviridae and cause Psittacine beak and feather disease (PBFD) in over 35 species of Old and New World Psittaciform. PBFD is chronic, progressive and irreversible viral disease. But it can cause severe, acute, disease syndrome in nestlings and African Grey parrots. Its clinical signs are symmetrical feather loss and dystrophy, and occasionally beak deformities. Avian polyomavirus is the causative agent of budgerigar fledgling disease (BFD). Its clinical sign is similar to PBFD. Alopecia is the representative clinical sign of BFD. Subcutaneous hemorrhage appear on nestling budgerigars. Aspergillosis is a fungal disease caused by fungi of the genus *Aspergillus*, in particular *A. fumigatus* and *A. flavus*: It has been described in many wild bird species, both in the natural and captive state. They make the spores, which are scattered in the environment and these spores penetrate aerial apparatus which has the ideal condition to germinate. Then they produce the vegetative forms and invade the tissue. The target organs are lung, and air sacs. But they can affect the liver, the kidney, the bones, the skin and the eyes.

**Materials & Methods:** African Grey Parrot became depressed two days ago, and the owner found yellowish diarrhea. These clinical signs suddenly appeared. And another clinical signs were alopecia in dorsal area and tail feather and paralysis of right leg. This bird died in few hours later despite of supportive care: fluid therapy, anti-bacterial drug, Vitamin B injection. For the accurate diagnosis about PBFD and BFD, the growing feathers were taken from the area of alopecia for PCR and autopsy was done. At autopsy, the fungal organism was detected in air sac of right thoracic area. To diagnose the accurate species of fungi, it was taken from the lesion, cultured in Sabouraud dextrose agar medium for PCR and smeared on the slide for microscopic examination.

**Results:** PCR result about growing feather from alopecia area indicated that PBFD was positive and BFD was weak positive. And fungal organism was identified as *Aspergillus fumigatus* by nucleotide sequence of PCR product about fungal organism.

**Discussion:** This case was mixed infection of PBFD, BFD and *Aspergillus* in African Grey Parrot. Aspergillosis was regarded as an immediate cause of death. Alopecia is clinical sign of chronic PBFD and BFD, so it can't make dead the bird suddenly. So, it was possible that the viruses, avian circovirus and avian polyoma virus, were infected the bird and made its immune system weak, then the spores of *Aspergillus* in the environment invaded the respiratory system and germinated in air sac. Alopecia is very common in bird these days and has many causes like viral disease, bacterial infection, fungal infection and ethological problem. PCR is very effective method to diagnose the causes of alopecia.

**Keywords:** African Grey Parrot, Psittacine beak and feather disease, Budgerigar fledgling disease, Aspergillosis

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#### A Subcutaneous Lipoma in a Male Red Fox (*Vulpes vulpes*)

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**Introduction:** Lipoma is a common benign tumor of well-differentiated adipocytes, which seen in most domestic animals. It is detected in diverse regions and the etiology for most lipomas is idiopathic. It was found in other species such as foals, guinea-pigs, leopards, swine, baboons, laboratory rats and prairie dog. This report describes lipoma in the cervical subcutaneous region of male red fox, for the first time.

**Materials & Methods:** An 8-year-old male red fox (*Vulpes vulpes*) in Species Restoration Technology Institute of Korea National Park Service (KNPS), revealed nodular growths in its ventro-cervical region. The fox was introduced from Young-Yang Gun in 2012 to KNPS for re-introduction of red fox. It has been cared in captive facility and showed the mass in August 2013 and was sent to Wildlife Medical Center. For the diagnosis of underlying disease and cervical mass, radiographical and sonographical examinations, complete blood count, serum chemistry analysis, peripheral blood smear examination and surgical removal of the mass were performed. The mass was fixed in 10% neutral buffered formalin and processed routinely for haematoxylin and eosin (HE) stain.

**Results:** Based on hematological and serum chemical examination, the fox showed slightly increase of the number of white blood cells, thrombopenia, increase of creatine kinase MB and uric acid. However, it was considered as no clinical relevance since the fox showed no related clinical signs. Macroscopically, the mass was round, whitish and well-demarcated. Microscopically, it was diagnosed as a lipoma consisting of mature adipose tissue. Lipoma is a common benign tumor in most domestic animals, however it has never been reported in the red fox.

**Discussion:** In summary, the present study is the first reporting of lipoma detection in red fox, although it has been found in many species and in many different forms. For the diagnosis of cervical lipoma in red fox, ultrasonography can be considered as a primary method, because it is non-invasive, economic and exact than others. However, for getting more exact diagnosis and knowing of physiological status, it is necessary to confirm the hematology and histopathology together.

**Keywords:** Lipoma, Ultrasonography, Red fox, *Vulpes vulpes*

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#### A Case of Multiple Trichoepithelioma in a Male Slow Loris at a Rescue Centre in Bogor, Indonesia

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#### Introduction

Slow lorises (Genus: *Nycticebus*) are nocturnal, arboreal prosimian primates that occur throughout Southeast Asia (Schulze, *et al.*, 2003). The greater slow loris (*N. coucang*) is found throughout the Indonesian Island of Sumatra and peninsular Malaysia. Although the conservation status of *N. coucang* is currently listed as Vulnerable (Roos, *et al.*, 2014), their numbers in animal markets far outstretch the ability of these slow-reproducing primates to recover their population numbers in the wild. Indeed, this threat raised international concern, resulting in the transfer of all members of the Genus *Nycticebus* to CITES Appendix I in 2007 (Nekaris and Nijman 2007). Since 2008, Yayasan IAR Indonesia has run the first specialized rehabilitation programme for the slow loris in Indonesia. Currently, more than 100 slow lorises are housed at the IAR rehabilitation centre, received from confiscations, or voluntarily surrendered by private owners (Citraningputri, 2011). This paper's objective is to overview the case report of a rare multiple trichoepithelioma in a male *N. coucang* in IAR Indonesia.

A variety of neoplasia have been reported in prosimians. Diagnosed tumours included hepatocellular carcinoma, adenocarcinoma, lymphosarcoma, fibrosarcoma and carcinoma of the thyroid, ovary and bile duct (Griner, 1983, Benirschke *et al.*, 1985, Lowenstine, 1986, Junge, 2003, in Streicher, 2004). Tumours of the skin and subcutis are also common among prosimians (incidence >15%), but only a few tumours were reported in slow lorises (Plesker *et al.*, 2002). Neoplasia was common in slow lorises in North American zoos during 1980-2010 where 17.4% of animals (n = 109) died or were euthanized owing to neoplasia during that time. However, none of these cases were of skin neoplasia (Fuller, *et al.*, 2010). Only one single case of a skin tumour in a greater slow loris has ever been reported (Lowenstine, 1986) and more recently one case of *trabecular trichoblastoma* in the neck of a pygmy slow loris (*N. pygmaeus*) (Plesker *et al.*, 2002).

#### Materials and method

A male slow loris (*N. coucang*) was received by IAR in May 2013 in very poor condition. This loris was thin, weak, shaking, had bone and articulation problems in both hands (probably hypocalcaemia) and also had 18 skin masses of various sizes (between 5 – 25 mm in diameter) located all over the body (hands, legs, back, stomach and forehead). During the first examination, we found that the masses were soft, very firmly attached to the skin, and revealed a cheese-like discharge when punctured. We isolated the discharge for bacterial culture and also took a biopsy of the masses for a histopathological examination. The result for the bacterial culture was *Staphylococcus aureus* and the histopathology result was a sebaceous gland adenoma. Since it was classified as a benign tumour, we decided to surgically remove the tumour. Following its removal, we conducted another histopathological check.

#### Result

Histopathological description:

The piece of tissue was part of a tumour that consisted of tumorous cells which were set in a cyst structure with various dimensions - the largest cyst was approximately 2mm. All the cysts were supported by myxoid stroma. Most of the cyst walls were formed by basaloid epithel cell with a minimum cytoplasm and hiperchromatic nucleus that made the cyst wall look blue. These basaloid epithel cells made the transition into the squamous epithel in a drastic way, forming "ghost cells" that were pale keratinized epithel cells with a blur nucleus. Mitotic were rarely seen. In most parts of the cyst lumen we also found a number of multifocal areas within the tumour.

The final result of the histopathological result was a trichoepithelioma. Since we found more than one tumour on the animal, we diagnosed it as multiple trichoepithelioma.

## Discussion

Trichoepithelioma is a benign follicular tumour composed of basaloid cells with follicular differentiation. In dogs and cats, spontaneous trichoepitheliomas are uncommon, occurring as small solitary tumours less than 2 cm in size. Multiple tumours and masses of up to 8 cm in diameter are even rarer (Goldschmidt & Hendrick 1998). In humans, two clinical forms are recognized: (1) multiple form (hereditary), display numerous skin coloured papules and nodules measuring less than 1 cm that develop on the face, scalp, neck and upper trunk during childhood. (2) Solitary lesions (non-hereditary), normally appear as pale or skin-coloured papules and nodules on the face of adults, sometimes reaching 2 cm in diameter (Murphy & Elder 1991).

Diagnosis of trichoepithelioma is based on the history, clinical examination and skin biopsy (Bozi and Katoulis, 2004). There was a similar case reported in humans where gross specimens of trichoepithelioma that contained cheese-like material within (Karikal et al, 2014). Histopathologically, trichoepithelioma vary considerably, depending the degree of differentiation and whether the tumour is primarily related to the follicular sheath or the hair matrix. Frequent characteristics include horn cysts, lack of intercellular bridges (desmosomes), differentiation toward hair-follicle like structures, formation of abortive or rudimentary hairs, desmoplasia, inflammation, melanisation and shadow (ghost) cells (Scott et al, 2001). Most trichoepithelioma case reports that featured histopathological descriptions reported the appearance of horn cysts, central keratin filled cystic areas lined with stratified squamous epithelium cells that had eosinophilic cytoplasm and a vesicular nuclei, basal cells with hyperchromatic nuclei and a granular layer, ghost cells and stroma ( Karikal et al, 2014, Mulas et al, 2006, Bozi and Katoulis, 2004, ).

Management of either form (i.e., solitary or multiple/hereditary) by superficial biopsy is usually adequate. Solitary lesions are treated by local excision (with a 2 mm margin) and cutting deep into the subcutaneous layer of the tumour. Multiple lesions may be left untreated but should be regularly monitored. However, it is best to remove all lesions if it is practical to do so (Karikal et al, 2013). In this case we have conducted about five excisions of tumours on the slow loris and so far there has been no sign of recurrence and the animal looks to be improving well.

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## Evaluation of Diagnostic Coelioscopy including Liver and Kidney Biopsy in Cinereous Vulture (*Aegypius monachus*)

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**Introduction:** The Cinereous vulture (*Aegypius monachus*) is one of the rarest species of raptors in the world. Globally endangered Cinereous vultures regularly migrate to South Korea for the winter, and they have been frequently rescued for various casualties. Endoscopy has been shown to be an effective diagnostic tool in veterinary medicine, and it also has been widely used in various species such as avian, reptiles and fish. A minimally invasive approach provided by avian endoscopy system allow detailed evaluation by providing visualization of the avian coelom and also facilitates the collection of tissue samples for histology or histopathology. The aim of this work was to investigate technique for diagnostic coelioscopy using the cinereous vultures as a large raptor model, the ability to visualize visceral structures, and assesses the technique of the liver and kidney biopsy through the coelioscopy.

**Materials & Methods:** Seven clinically healthy cinereous vultures were anesthetized with isoflurane for the left approach coelioscopic examinations. The 5 mm rigid telescope system was used for the examination of coelomic viscera. Ease of entry and coelomic viscera visualization were scored, and liver and kidney biopsy samples were harvested from the four cinereous vultures by use of 5 mm (15-French) endoscopic biopsy forceps. Diagnostic quality of the biopsy samples were scored as poor, good, or excellent.

**Results:** For all vultures, mean entry and visualization score of the diagnostic coelioscopy were satisfactory to excellent for all measured parameters except esophagus and pancreas. The coelioscopic examination of coelomic viscera and biopsy of liver and kidney were safe, simple to accomplish with appropriate equipments, and harvested tissue samples suitable for histologic and histopathologic test.

**Discussion:** The minimally invasive coelioscopy using the 5 mm telescope system is safe and effective procedure for visceral examination of the large raptors such as cinereous vultures, and the coelioscopic biopsy is recommended for the collection of tissue samples that are suitable for histologic and histopathologic interpretation.

**Keywords:** *Aegypius monachus*, Cinereous Vulture, Coelioscopy, Liver, Kidney, Biopsy

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## Standing Anaesthesia for Molar Incision in Sumatran Elephant (*Elephas maximus sumatranus*)

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One Female sumatran elephant showed abnormal behaviour by frequently insert its trunk to the mouth and decreasing appetite. Physical examination found that its upper molar failed to replace and pushed by the new molar, an incision made to reduce pain pressure of the upper molar to the surround mucosa by using the standing anaesthesia method with ketamine and xylazine. The incision was made between or intra laminae. Modified mouth gag use to remain the mouth open during incision.

**Keyword:** Sumatran elephant molar incision, standing anaesthesia, modified mouth gag

**Introdution:** Elephant's mouth is such an enviromentally sensitive and dynamic process. The rate of eruption, wear and loss of the elephant's molar teeth rarely occurs in the same identical time frame on both side of the mouth. Asymmetric change gear on an elephant can cause changes in the clinical signs of elephants, for examples is the decrease in appetite due to pain because of the failure of the old molar apart. Several preventive methods by providing hardwood steems is done to eliminate molar problems in elephant. An incision is made to reduce pain and stop the abration in the mucosa of mouth. These procedure considered to be a minor surgery with standing anaesthesia procedures.

**Signalement:** Sumatran elephant name Deti showed abnormal behaviour. Deti often seem inserting its trunks to its mouths. Elephant keeper also notified that deti has reduced in appetite, and less activity. Physical examination showed that deti's right upper molar pressing the surrounding mucosa and cause injury. The right upper molar is longer that the left side (Figure 1)

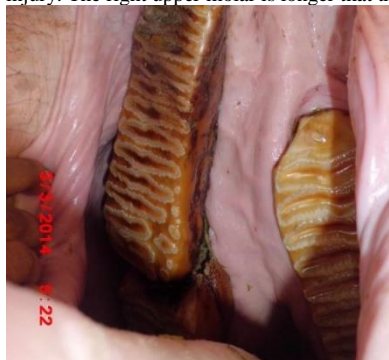


Figure 1. Deti's unsymetrical upper molar

**Material and Methods:** A 30-years-old female Sumatran elephant was anesthezied using the combination of Xylazine and Ketamine. 0.1mg/kgBW of Xylazine mix together wit 0.1mg/kgBW of Ketamine and delivered together in one syringe. The drugs reactions is about 15-20 minutes. Anesthezied elephant will laid it trunk in the ground, and sleppy eyes (Figure 2). And intravenous catheter with lactated ringer's fluid placed in the medial of ear to maintain the depth of anaesthesia and provide intravenous medication.

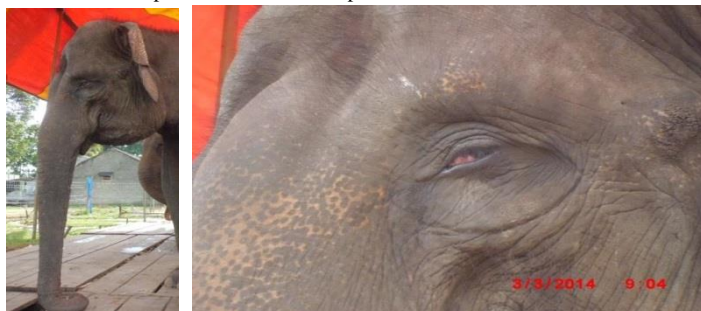


Figure 2. Standing anesthesia in Elephant

Modified mouth gag (Figure 3) placed around the trunk and lower lips (Figure 4) to ensure the mouth open during the procedure the incision takes place so as not to injure the mucosa. Incision was made between ridges or intralaminae using electric grinding machine. The depth of incision is about 4 cm. Every sharp edges made by the incision procedures must be smoothed to avoid further injury. (Figure 4)



Figure 3. Modified mouth gag for elephant and hand grinding machine



Figure 4. Elephant mouth gag placement

### Results

Unsymetrical molar that caused mouth pain and mucosal injury can be treated by doing incision in the part of elongated molar (Figure 5). Standing anesthesia procedure can be done in elephant, combining with modified mouth gag for elephant, the procedure can be done by avoiding general anesthesia or laying anesthesia risk.





Figure 5. Incised molar

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### Acute Intoxication of Zinc Phosphide in a Wild Sumatran Elephant (*Elephas maximus sumatranus*) in Bukit Tigapuluh Landscape

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**Abstract:** An adult female Sumatran elephant (*Elephas maximus Sumatranus*) was died in Bukit Tigapuluh landscape. Overall the carcass condition was good. Blood fluid issued from mouth cavity. Rectum and vulva was prolapsed bleeding. There was blood clotting on muscle incision. The peritoneum was black on the right lateral area. On toxicology test, positively high ammonia and zinc phosphide were found on gastric juice sample.

**Key words:** Sumatran elephant, zinc phosphide, intoxication

**Introduction:** Zinc phosphide is an inorganic chemical that is used to be as rodenticide formed in black powder (Johnson and Fagerstone, 1992). Zinc phosphide reacts in the stomach and intestines with water and hydrochloric acid to liberate phosphine gas to cause severe irritation. The phosphine gas can enter the blood stream and adversely affect the lungs, liver, kidneys, heart and central nervous system. Symptoms of acute zinc phosphide poisoning include nausea, shock, weak heartbeat, low blood pressure and unconsciousness (Clarkson, 1991). Other symptoms include vomiting, diarrhoea, cyanosis, restlessness and fever.

**Materials and Methods:** A 25-years old female Sumatran elephant (*Elephas maximus Sumatranus*) died on buffer area of Bukit Tigapuluh National Park, Jambi, Indonesia in late December 2013. The necropsy was executed three days in estimation after death. Overall the carcass condition was good and has not autolysis post mortem yet. The representative samples were sent to laboratory for histopathological and toxicology examination.

**Results:** On external examination, there was no any depression or contusion. Blood fluid issued from mouth cavity. Rectum and vulva was prolapsed bleeding. It might due to severe colic before she died. There was blood clotting on muscle incision. The peritoneum was black on the right lateral area. When it was incised, there was black fluid looked like asphalt issuing. In abdominal cavity, there was approximately five litres of situsviserum which means she had hidrops ascites. The intestines contained fewer amounts of feed and faeces. Surprisingly, we found plastic package of pesticide in the middle of it. The hay around that plastic was black coloured. In toxicology examination, positively high ammonia and zinc phosphide were found on gastric juice sample.

**Discussion:** Haemorrhage, blood clotting within the carcass, hidrops ascites and indigestible feed on stomach showed that the death was acute (Cooper and Cooper, 2007). The elephant died just several time after meals due to its indigestible feed. The pesticide plastic contained zinc phosphide leads to haemotoxicity which cause haemolysis within the body, showed as haemorrhage, blood clotting and hydrops ascites.

**Conclusion:** Based on physical, histopathological and toxicology examinations, the elephant was died due to acute toxicity of zinc phosphide.

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## INFECTIOUS DISEASE

### Advancing Wildlife Disease Surveillance in Vietnam through Cross Sector Collaboration

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#### Abstract:

Human population growth and subsequent expansion into wildlife habitat combined with an increase in hunting, long distance trade and consumption of wildlife is thought to be driving the emergence of infectious disease. Approximately 75% of all new, emerging and/or re-emerging diseases which affect humans at the beginning of the 21<sup>st</sup> century are sourced from animals, and of these, 72% originate from wild species. Both the severe acute respiratory syndrome (SARS) virus outbreak in 2003 and the on-going Ebola outbreak in West Africa today, are examples of diseases in humans that have emerged as a result of spillover from a wild population and human behaviors associated with wildlife trade and consumption. Identifying pathogens of pandemic potential in wildlife BEFORE they spillover into humans or domestic animals or RAPIDLY once a spillover event has occurred, is considered critical to the global effort to reduce the emerging pandemic threat. Early detection will only be possible if the capacity for surveillance at the human/wildlife/domestic animal interface is present in countries like Vietnam considered “hot spots” for disease emergence.

The PREDICT project<sup>1</sup> was designed to build a global early warning system of zoonotic diseases that can move between wildlife and humans. In Vietnam the first stage of our extensive study identified potential high-risk interfaces for zoonotic disease spillover from wildlife to humans. Many of the high-risk interfaces identified in Vietnam were linked to the domestic and international trade of wildlife. Through a collaborative effort with Vietnamese research, academic and government institutions the project established the local capacity to conduct disease surveillance and pathogen detection in wildlife at these interfaces. Samples were opportunistically collected from illegally traded wildlife seized by enforcement agencies and wildlife from restaurants, wildlife farms, markets and rescue centers. Field surveillance capacity building covered the safe handling of wildlife, use of personal protective equipment (PPE) and the collection, storage and transport of high quality samples for diagnostic investigation. Samples were tested to identify novel viruses using consensus conventional polymerase chain reaction (PCR) which is a broadly reactive diagnostic assay designed to identify all members (known and new) of a given viral family. Samples were screened for viral families known to cause disease in humans including Alpha-, Arena-, Bunya-, Corona-, Filo-, Flavi-, Hanta-, Henipa-, Herpes-, Influenza, Paramyxo-, Rhabdo- and Seadorno- families/genera. PCR products were then cloned and sequenced. The

sequences were then compared to known viruses within a family/genus. This general diagnostic approach is not limited to samples of wildlife origin and can be applied to disease outbreaks and clinical investigations in domestic animal and human populations. PREDICT wildlife surveillance efforts in Vietnam involved the collection of 6,500 samples from over 2,000 individuals at 10 distinct human/wildlife interfaces across the country. Over 15,000 tests were performed and multiple new and known viruses were detected in wild carnivores, rodents and bats. The results contribute to increasing our understanding of the pathogens present in wildlife populations at high risk interfaces where there are opportunities for pathogen spillover to humans or domestic animals. The established capacity for surveillance and pathogen detection in wild populations in Vietnam will contribute to efforts focused on detecting and containing emerging disease including providing the information necessary to reduce wildlife consumption and trade through changes in policy and human behavior. The field-based surveillance and laboratory diagnostic protocols can also be used in wildlife conservation efforts to screen wildlife in restoration and release programs and reduce the risk of introducing new pathogens back into wild populations.

### Molecular Characterization of *Mycobacterium orygis* Isolates from Wild Animals of Nepal

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#### Abstract

##### Introduction

*Mycobacterium tuberculosis* complex (MTC) species cause tuberculosis (TB) in humans and animals. Recently, *M. orygis* has been considered as a distinct member of MTC. We have isolated this bacterium from a spotted deer (*Axis axis*) and a blue bull (*Boselaphus tragocamelus*) in Nepal.

##### Materials and Methods

During postmortem, TB suspected lesions from lungs and lymph node in deer and only lungs in blue bull were collected and cultured in LJ media. Spoligotyping, mycobacterial interspersed repetitive units-variable number of tandem repeats (MIRU-VNTR), region of difference analysis (cfp32, RD9 and RD12) and SNP detection were performed to ascertain species and molecular epidemiology of the isolates.

##### Results and discussion

Both isolates had a typical spoligotype, SIT587 in SpolDB4 database, corresponding to *M. orygis* and in an agreement with many other reported isolates. RD analysis (cfp32: +, RD9 - and RD12-) and SNP detection of *gyrB*, *mmpL6*, *TbD1*, *PPE55* and *Rv2042c* confirmed the isolate to be *M. orygis*. Also, in MIRU-VNTR analysis, these isolates had a similar pattern as of many other reported isolates. *M. orygis* have been isolated from many animals and humans of South Asia. This finding may indicate its endemic prevalence in the sub-continent. Identification of a new member of MTC in Nepal may reveal the complexity of tuberculosis in the country. Further investigation including increased sample size and different host interaction will help to understand the ecology and epidemiology of *M. orygis* in Nepal.

##### Conclusion

*M. orygis* was isolated for the first time in Nepal. The finding can be helpful to understand the situation of tuberculosis in the country.

##### Keywords

*Mycobacterium orygis*, Spoligotyping, Mycobacterial interspersed repetitive units-variable number of tandem repeats (MIRU-VNTR), Single nucleotide polymorphism (SNP)

### Surveillance of Disease in Wild-mammal in Gangwon Region

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**Abstract:** Diseases carried by wild animal have been increased, therefore concern for domestic disease and zoonosis from wild animals has been increased. In this study, 154 wild mammals of 3 species were monitored for rabies, tuberculosis, brucellosis, Bovine Viral Diarrhea, canine distemper, Aujeszky's disease and Foot and Mouth Disease in Gangwon region. Tissue samples were used for antigen detecting test. Serums were also used for antibody detecting test. Results of tests for all diseases were negative. The continuous monitoring of diseases in wild animal will be used for data base for epidemiologic study and the prevention of disease; Key Words: Monitoring, Epidemiologic survey, Zoonosis, Wild animal, Domestic animal disease

**Introduction:** Due to global warming and changes in distribution and the number of wild animals, diseases carried by wild animals has been increased. Disease monitoring in wild animals is concerned with epidemiological survey of infectious diseases, therefore there are many studies in progress. The density of wild animals in Gangwon region are especially higher than in other areas, therefore there is a need for continuous monitoring and survey. In this study, we investigated infections of water deer, roe deer and raccoon in Gangwon region in order to monitor for zoonosis and diseases in domestic animals.

**Materials & Methods:** Wild mammals were collected from August 2013 to July 2014 in Gangwon region. Total of 154 wild mammals of 3 species (Water deer 107, Roe deer 6, Raccoon 41) were transferred and examined for monitoring. The animals were monitored for rabies, tuberculosis, brucellosis, bovine viral diarrhea (BVD), canine distemper, Aujeszky's disease and foot and mouth disease (FMD). Tissue samples (intestine, lymph node, spleen) were used for antigen detecting tests for BVD, brucellosis and tuberculosis, and brain tissues of raccoons were tested for rabies and canine distemper. Serums were also used for Rose-Bengal test, kit test and ELISA to check the antibody of brucellosis, Aujeszky's disease, tuberculosis and FMD.

**Results:** ELISA was conducted for FMD, tuberculosis, Aujeszky's disease of 107 water deer and 6 roe deer. The results of the ELISA tests were negative. The results of Rose-Bengal test for brucellosis and the results of Antigen detecting test for tuberculosis, brucellosis and BVD were also negative. Results of antigen detecting test and ELISA in 41 raccoons were negative. Canine brucellosis kit was used for detecting antibody for brucellosis of raccoon, and the results were negative. Results of antigen and antibody detecting test for rabies, tuberculosis, brucellosis, BVD, canine distemper, Aujeszky's disease and FMD were identified as negative for all diseases.

**Discussion:** The continuous monitoring of diseases in wild animal will be used for base data for epidemiologic study and the prevention for domestic animal disease. This will contribute to blocking outbreak and spread of diseases which threat people and domestic animals

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## Monitoring of Chronic Wasting Disease in Water Deer (*Hydropotes inermis argyropus*) in Gangwon Province, South Korea

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**Abstract:** The three hundred two samples (79 Brain, 85 laryngopharyngeal lymph node, 85 tonsil, 53 rectal mucosa) of 86 free-ranging water deers in Gangwon province were examined for CWD detection in this study. Prion Rapid test kit (Bio-Rad TeSeE™ SAP Combi Kit) was first used for screening, and if kit was positive, re-test was progressed. If re-test resulted positive, performed western blotting and immunohistochemistry for exact diagnosis. As a result, there wasn't conformed CWD infection in wild water deer in Gangwon province; Key Words: Chronic wasting disease, water deer, Gangwon, Prion disease

**Introduction:** Chronic wasting disease (CWD) is one of prion diseases such as Bovine Spongiform Encephalopathy (BSE) or variant Creutzfeldt-Jakob disease (vCJD), and affects cervids. There has been no report on human infection, but there has been several reports on experimental infection of cattle, sheep, monkey, mouse, etc. CWD has been detected in wild and farmed cervids in the U.S.A and Canada. Korea also has reported several outbreaks in captive cervids. Since 2001, after the first detection of CWD from imported captive mule deer, Korea has been thoroughly monitoring captive deers, but wildlife animals should also be monitored because they can be a vector for infectious disease in domestic animals. The water deer (*Hydropotes inermis*) is a small deer who natives to China and Korea. There are nearly 600,000 water deers in Korea. Monitoring of CWD in water deers would help prevent infectious disease in domestic animals and zoonoses.

**Materials & Methods:** 302 tissue samples (Brain: 79, Laryngopharyngeal lymph node: 85, Tonsil: 85, Rectal mucosa: 53) of 86 free-ranging water deer in Gangwon province were examined for CWD detection. Water deers were collected by Gangwon wildlife animal rescue center. Each sample was tested with Prion Rapid test kit. The kit was first used for screening, and if positive, immunohistochemistry and re-test were progressed. If re-test resulted a positive result western blotting was performed for exact diagnosis.

**Results:** The majority of tested water deers were transferred to Gangwon wildlife animal rescue center due to traffic accident. There were much more males than females. Most of them had fractures or external injuries. There was no CWD positive in 302 tested samples in Prion Rapid test kit, so re-test, immunohistochemistry and western blotting were not necessary.

**Discussion:** In this study, there was no CWD infection reported in wild water deer in Gangwon province. There were several CWD infection cases reported in domestic deers in South Korea, but not in Gangwon province. However, since there were CWD infected deer near Gangwon, Gangwon province is still potential to CWD infection. Though water deers are the majority of the deer population in South Korea, there are still other types of deer species. It is necessary to survey other cervids such as roe deers. Continuous monitoring of wildlife cervids will prevent infectious disease in domestic animals and zoonoses, and moreover contribute to veterinary public health.

**Acknowledge:** This work was supported by Eco-Innovation 416-111-012, Korea.

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## Monitoring of Wild Mammals for *Mycobacterium bovis* in Gangwon Province

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**Abstract:** Bovine tuberculosis is a disease from *Mycobacterium bovis* infection, and it is a zoonosis which can affect human. The purpose of the research is monitoring *M. bovis* infested wild animals in Gangwon province through serological test and bacteriological cultivation. One raccoon had positive result in rapid kit test, however it had no positive results for *M. bovis* in the cultivation and PCR test. In this study, *M. bovis* was not found in all samples, however there is a high risk of wild animal *M. bovis* infection and spread to livestock. Continuous and systematic *M. bovis* researches and blocks are necessary; Key words: Bovine tuberculosis, *Mycobacterium bovis*, Wild mammals, Gangwon province, Zoonosis

**Introduction:** Bovine tuberculosis is a disease from *Mycobacterium bovis* infection, and it is a zoonosis which can affect human. *M. bovis* is a part of mycobacterium tuberculosis complex and its hosts are wide and various wild animals. There is a high chance that wild animals infested *M. bovis* may spread infection to livestock. The purpose of the research was to monitor *M. bovis* infested wild animals in Gangwon province through serological test and bacteriological cultivation.

**Materials and Methods:** 109 free-ranging wild mammals of 3 species which were transferred to Kangwon National University Wildlife Medical & Rescue Center from November, 2013 to July, 2014 were tested. In order to detect *M. bovis*, whole blood and serum were collected for rapid kit testing and tissue samples were used for bacterial isolation with Lowenstein-Jensen, 7H11 media and mycobacteria growth incubator tube (MGIT) and PCR technique.

**Results:** One raccoon had positive result in rapid kit test, however it had no positive results for *M. bovis* in the cultivation and PCR test. There was no positive results in *M. bovis* in culture media, whereas several non-tuberculosis mycobacterium were isolated. Two raccoons were detected for *Mycobacterium avium* and *Mycobacterium intracellulare* was found in 5 water deers and one raccon. 15 water deers and 3 raccons were detected for other nontuberculous mycobacterium.

**Discussion:** Although no human infection for *M. bovis* has been reported in South Korea, infection to human has been reported outside of South Korea. Researchers consider *M. bovis* to be highly contagious from wild animals to livestock and humans. In this study, *M. bovis* was not found in all samples, however there is still a high risk of wild animal *M. bovis* infection and spread to livestock. Continuous and systematic *M. bovis* researches and blocks are necessary. This study presents possibilities about *M. bovis* contagion from wild animals to livestock and human and will be used for basic data for bovine tuberculosis eradication and precautions.

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## Histopathological Evaluation of Natural Case of Highly Pathogenic Avian Influenza Subtype H5N8 in Chicken, Korea

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**Introduction:** In 2014, H5N8 HPAI viruses were isolated from Baikal teals that were found to be 100 carcass (A/Baikal Teal/Korea/Donglim3/2014), a breeder duck farms (A/breeder duck/Korea/Gochang1/2014), and broiler duck farm (A/duck/Korea/Buan2/2014). Start with those cases, there are many

outbreaks caused by H5N8 HPAI virus including our case in 2014 winter. However, it remains unknown that that detailed pathobiology of H5N8 HPAI, such as host adaptation, tissue tropism, pathological lesions, infectivity and transmissibility is unclear until now. This report is the first description of natural cases with clinical and pathological findings of broiler breeder and commercial layer chickens infected with H5N8 HPAI.

**Cast History:** On February 26, veterinarian submitted 5 dead broiler breeders (20-week-old) to the Avian Disease Laboratory, College of Veterinary medicine, Chungbuk National University, South Korea. On February 18, the birds were moved to laying house from the pullet house at the age of 139 days old. And then, the mortality at the breeder's farm, which housed 15,000 birds, began with 60 birds on February 22. From the day after mortality peak, mortality had been gradually decreased (by 100, 70, and 60, each day) until the submission of samples for diagnosis. Based on the mortality and gross pathological findings, this case had been tentatively diagnosed as "fowl typhoid" by practitioner and there were no specific respiratory sign, diarrhea, and lameness. On March 13, the outbreak occurred on a commercial layer farm with 3 total barns including house A, house B, and house C. The mortality was noted in the only one flock in house C. The flocks consisted of about 8000 hens that had not present noticeable clinical signs. The mortality was slightly increased (0.1-0.3% mortality for first three days) at the back of 4, 5, and 6 rows. Although this mortality was not indication of significant infectious diseases, the farmer sent this case to our laboratory because of the pattern of mortality. Actually, the farmer suspect this case as fowl typhoid like broiler breeder case described above. On March 15, the mortality was also occurred in of 1, 2, and 3 rows (1-2 dead birds per one cage). All birds and their eggs of both broiler breeder farms and commercial layer farms were buried immediately after officially diagnosing by Avian Disease Division, Animal and Plant Quarantine Agency (Anyang, South Korea).

**Results:** There are prominent congestions of all tissues. No significant lesions are present in the proventriculus, gizzard, and intestines. In respiratory system, there are mild exfoliation of tracheal epithelia and focal edema of lungs. The mild periportal aggregation of mononuclear inflammatory cells is present in the liver with necrosis of inflammatory cells. There are severe congestions of renal interstitium with multifocal tubular necrosis. The multifocal or diffuse necrosis is found in the ovary, spleen, and pancreas. Besides, in spleen, generalized depletion of lymphoid cells is observed. In brain, the generalized congestion, multifocal malacia, gliosis, and mild perivascular cuffing are mainly found in cerebrum. In some regions of brain, central chromatolysis of neurons is observed simultaneously. The similar histopathological lesions are found in various tissues of commercial layer such as diffuse necrosis of hepatocytes and pancreatic acinar epithelium, dissociation of muscle fiber in heart, and multifocal malacia and mild perivascular cuffing in brain. In many visceral organs, including, lung, liver, spleen, ovary and intestine, moderate to intense influenza viral nucleoprotein-immunopositive cells were found in submucosa or interstitium. The morphology of influenza viral nucleoproteins-immunoreactive cells is assumed to be mononuclear phagocytic cells. Some tubule epithelium of kidney and acinar epithelium of pancreas were occasionally immunostained for influenza viral antigen. The influenza viral antigen was mainly detected in brain. In cerebrum, the nucleus and cytoplasm of pyramidal cells and glial cells were positive for influenza viral antigen. In the cerebellum, influenza viral nucleoproteins-immunoreactivity was observed in nucleus or cytoplasm of some purkinje cells and glial cells in molecular layers and granular layers.

**Discussion:** In this study, using pathological examination and immunohistochemistry, it has demonstrated that H5N8 NPAI isolated from two natural cases shown a general tissue tropism. During the assembly stage of viral replication, the haemagglutinin proteins are cleaved and infected to the cells and this allows the HPAI virus to replicate in various tissue types. As seen this case, the influenza viral antigens were mainly localized in ependymal cells of ventricles. This would be the possible infection route of H5N8 HPAI viruses. As seen in our results, broiler breeders and commercial layers infected with H5N8 HPAI were represented the similar pathologic lesions and distribution of influenza viral antigen to previous H5 HPAI outbreaks. However, this H5N8 HPAI outbreak has some differences to the H5N1 HPAI that occurred in South Korea in 2003-2004. According to flock history of both cases, the mortality had started suddenly, and then number of dead birds has dwindled away. This means that there are some "self-limiting" mechanisms of viral replication and slow transmission regardless of floor or cage-rearing house system. The differences in mortality patterns had been also caused by age or immune status of the infected host, the route of exposure, and the host adaptation is the crucial in beginning the process of HPAI infection. To clarify this unusual pattern of mortality, further studies will be likely required to understanding of pathobiology H5N8 HPAI viruses and dealing with the threat to economic loss and public health caused by HPAI.

**Keywords:** H5N8, Avian Influenza, Pathology, Chicken

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### The Immune Response of Red Jungle Fowl to Newcastle Disease Vaccines, Lasota and Mukteswar Strains

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**Abstract:** This study evaluated the immune response of red junglefowl raising at Cuc Phuong National Park to Newcastle disease vaccines. The results showed that all animals in the experiment well responded to the vaccines. After the first dose with LaSota strain (7 days old), the antibodies titer gradually raised to the highest of 4.5 log<sub>2</sub> (21 days post vaccination, DPV) and dropped bellowing the protective level of 3 log<sub>2</sub> (28 DPV). Upon the second dose with LaSota strain (28 days old) and booster with Mukteswar strain (45 days old), all of animals developed a firm immune response, and retained above the protective titer for at least 35 days.

**Key words:** Red junglefowl, immune response, Newcastle disease vaccine

**Introduction:** The red junglefowl (*Gallus gallus spadiceus*) has been raised and multiplied in Cuc Phuong National Park since 2007. And the prevention measures against infectious diseases are demanded. This study provided data of the immune response of red junglefowl to Newcastle disease vaccines, and helped to design a suitable vaccine schedule.

**Materials & Methods:** Red junglefowl has not been vaccinated by Newcastle disease vaccine. Vaccines used were either LaSota or Mukteswar strains. The antibody titer of chickens post vaccination was evaluated by a standard hemagglutination inhibition (HI) test [1].

**Results:** After primary immunization with LaSota strain, at 7 DPV, there were 86.70% of vaccinated chickens having the antibody titer  $\geq 3 \log_2$ . The titer raised gradually, was above the protective level (3 log<sub>2</sub>) at 21 DPV, then it dropped at 28 DPV. It was demonstrated that the second shot with LaSota strain invoked higher level of immune response. As such, the antibodies titer of chickens was above the protective titer, and maintained at least to 28 DPV. A solid immunity against Newcastle disease virus was obtained by a booster with Mukteswar strain. Of which, the titer was always  $> 3 \log_2$  for at least 35 DPV, got peak (6.1 – 6.3 log<sub>2</sub>) on 28 DPV.

**Discussion:** This study showed that the red junglefowl responded properly with the Newcastle disease vaccines. It suggested that the combination of LaSota and Mukteswar strains should be used for immunization protocol.

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## Prevalence of Antimicrobial-resistant *Escherichia coli* from Okinawa Rails (*Gallirallus okinawae*) and Overlap of their Habitat with Human Habitation on Okinawa Main Island

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**Introduction:** The Okinawa rail (*Gallirallus okinawae*) inhabits the evergreen laurel forest zone called “Yanbaru”, located in the northern part of Okinawa Main Island, Japan. Although this species was historically distributed across a broad region of the island, its population size has decreased due to deforestation and predation pressure by the small Indian mongoose (*Herpestes auropunctatus*), an invasive alien species. Therefore, the Okinawa rail is listed as an endangered species on the IUCN Red List of Threatened Species and is also designated as a natural treasure of Japan. However, the population of this species has recently increased around the sphere of human habitations, such as villages and cattle farms, where the rails can easily obtain food. However, the direct and/or indirect contact between wildlife, livestock and humans also causes several problems such as transmission of infectious diseases and of antimicrobial-resistant bacteria (ARB). The objectives of this study were to clarify the invasion of antimicrobial-resistant *Escherichia coli* among Okinawa rails and across the human habitations where this species uses in order to evaluate the health status of the “Yanbaru” ecosystem from the perspective of conservation medicine.

**Material and methods:** Fecal and environmental samples were collected from the prefectural road and the forest of the Kunigami District of Okinawa Main Island (26°47'33", 128°18'58.1"). One hundred twenty-six fecal samples of Okinawa rails and 36 environmental samples (23 soil samples and 13 water samples) were collected from human habitations and cattle farms where Okinawa rails were frequently observed. Thirty-three fecal samples of Okinawa rails and 45 environmental samples (31 soil samples and 14 water samples) were collected at sites far from human habitations, including cattle farms as controls. Samples were collected between June 2012 and May 2014 and were transported to our laboratory in a refrigerated state. *Escherichia coli* was isolated from fecal samples of Okinawa rails using routine culture methods. Colilert™ (IDEXX Laboratories, Inc., Westbrook, Maine, USA) was used for isolation from environmental samples. The standard Kirby-Bauer disk diffusion method was used to determine the antimicrobial agent sensitivity profiles of *E. coli* isolates against 17 antimicrobial agents.

**Results & Discussion:** *E. coli* was isolated from 65.1% of 126 fecal samples of Okinawa rails and from 36.1% of 36 environmental samples from human habitations and cattle farms where Okinawa rails were observed. Among the *E. coli*-positive samples, 84.1% of 82 fecal samples and 84.6% of 13 environmental samples showed antimicrobial-resistance. In contrast, the prevalence of *E. coli* was 63.6% among 33 fecal samples from Okinawa rails and was 24.4% among 45 environmental samples collected from sites far from human habitations. Antimicrobial-resistant strains were found in 23.8% of 21 *E. coli* strains from fecal samples and in 9.1% of 11 *E. coli* strains from environmental samples far from human habitations. Previous research revealed that antimicrobial-resistant *E. coli* strains were found in 12.5% of wild Green pheasants (*Phasianus versicolor*) and in 15.8% of wild Bamboo partridges (*Bambusiothraupis thoracicus*) in Japan. Compared with these reports, the prevalence of ARB was the highest in Okinawa rails that use human habitations. The prevalence of ARB in the fecal and environmental samples from the areas far from the villages and cattle farms was significantly lower (by Chi-square analysis,  $p < 0.05$ ) than that of samples obtained from human habitations. Three types of multiple resistance patterns were detected in both Okinawa rails and environmental samples, one of which was collected from sites far from human habitations. In addition, eight types of multiple resistance patterns were detected from Okinawa rails from the human habitations, including a cattle farm. These results indicate that ARB have been transmitted from human habitations to Okinawa rails. On the other hand, as ARB were also detected from the environments far from human habitations, which suggests that Okinawa rails could spread ARB across the “Yanbaru”, their natural habitat. We conclude that it is necessary to plan an effective conservation strategy for the Okinawa rail from the perspective of environmental and ecological health.

**Keywords:** Okinawa rail, antimicrobial-resistant *Escherichia coli*

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## Prevalence of *Salmonella* in the Green Anole (*Anolis carolinensis*), an Invasive Alien Species: Okinawa Main Island 2009-2013

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**Introduction:** The green anole (*Anolis carolinensis*) is a lizard native to North and Central America. Its distribution has also spread to several non-native countries, where it is sometimes invasive. In Japan, the green anole was assumed to have been introduced to the Chichi and Haha Islands of the Ogasawara Archipelago in the 1960s and 1980s. In addition, it was recently found to have colonized the Okinawa Main Island in the 1990s. Previous studies have shown that green anoles could carry *Salmonella*, and a high prevalence of this bacterium was observed in the population of green anoles on Guam and in the Ogasawara Archipelago. However, very few studies have investigated the *Salmonella* infection rates of green anoles on Okinawa Main Island, where the species has been more recently introduced than other areas. The aim of this study was to investigate changes in the prevalence of *Salmonella* in green anoles on Okinawa Main Island over 5 years to understand the dynamics of *Salmonella* infection and to contribute to risk evaluation and management of this pathogen.

**Materials & Methods:** From July 2009 to August 2013, 608 green anoles were captured by a company delegated by the Ministry of the Environment in residential areas and city parks near the Japan Ground Self-Defense Force's Camp in Naha, Okinawa. The green anoles were frozen at -30°C after capture and shipped to our laboratory. Frozen green anoles were thawed at room temperature and their intestinal contents were extracted for further testing. *Salmonella* was cultured and serological typing was performed using routine methods. Susceptibility to 12 types of antibiotics was also tested using the disk-diffusion test (Kirby-Bauer Method).

**Results:** *Salmonella* spp. were isolated from 3.9% of the 178 green anoles captured in 2009, 1.0% of the 100 captured in 2010, 2.0% of the 100 captured in 2012, and 4.0% of the 100 captured in 2013. *Salmonella* was not isolated from the anoles captured in 2011. The *Salmonella enterica* serovar Weltevreden was isolated from 8 strains in 2009, 2010, 2012, and 2013, and the serovar Enteritidis was isolated from 6 strains in 2009, 2012, and 2013. Two of the *Salmonella* strains detected in 2009 belong to *Salmonella* Enteritidis, and both showed resistance to oxytetracycline.

**Discussion:** The prevalence of *Salmonella* did not significantly increase during the 5 years of the study. In Guam and the Ogasawara Archipelago, the prevalence of *Salmonella* in green anoles has been reported to be high, and *Salmonella* spp. have been isolated from soil, stools of wild animals, and public toilets. It has been suggested that the habitat of the green anole and reservoir animals could also be contributing sources of *Salmonella* infection in green anoles. On Okinawa Main Island, we suspect that such interactions between green anoles and other infected animals and/or environments have not yet occurred. The *Salmonella enterica* serotypes Weltevreden and Enteritidis that were isolated from 14 green anoles have also been identified in cases of bacterial food poisoning in Okinawa. The identification of oxytetracycline-resistant *Salmonella* in green anoles indicates that they may have become infected through exposure to human living environments, because oxytetracycline is widely used as a human and animal medication and as a livestock feed additive. We conclude that green anoles may not only play an important epidemiologic role in human health as a reservoir of *Salmonella* but may also suffer from human activities. Therefore, in order to control *Salmonella* infection via green anoles, the interactive role of infection between human environments and wild animals regarding ecological health must be understood.



**Keywords:** green anole, *Anolis carolinensis*, *Salmonella*, invasive alien species, lizard, reptiles

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**PARASITOLOGY**

**Prevalence and Molecular Phylogeny of Avian Malaria Parasites in Columbiformes in Japan**

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**Introduction:** Avian malaria parasites (*Plasmodium* spp. and *Haemoproteus* spp.) and closely related parasitic *Leucocytozoon* spp. are observed in a variety of pigeons and doves (order: Columbiformes) worldwide. To date, these blood parasites have been found in 11 species and 13 subspecies belonging to 6 genera of the order Columbiformes in Japan (Murata, 2002; Yoshimura *et al.*, 2014; Hagihara *et al.*, 2005). Although mtDNA sequence data of *Haemoproteus* spp. from the Oriental turtle dove (*Streptopelia orientalis*) in Hokkaido has been deposited in GenBank, the prevalence and molecular phylogeny of avian malaria parasites and *Leucocytozoon* spp. from pigeons and doves inhabiting Japan are still not known. This study clarified the prevalence of avian malaria parasites in 6 species belonging to the order Columbiformes in Japan and estimated their molecular phylogeny.

**Materials & Methods:** Blood samples were collected from 14 Japanese green pigeons (*Treron sieboldii*), 33 Japanese wood pigeons (*Columba janthina*), 65 Oriental turtle doves (*Streptopelia orientalis*), 3 Grey-capped Emerald doves (*Chalcophaps indica*), 16 Taiwan green pigeons (*T. formosae*) and 26 rock doves (*Columba livia*). These birds were either caught in the wild or were under protection/protective captivity in animal hospitals or zoological gardens because of injury or disease during a 9-year period from 2004 to 2012. The partial cytochrome *b* (*cytb*) gene (478 bp) of the avian malaria parasite mitochondrial genome was amplified by nested polymerase chain reaction (nested PCR). The same procedure was used for *cytb* of *Leucocytozoon* spp. (Hellgren *et al.*, 2004). The PCR products were directly sequenced in both directions. The sequences were compared with GenBank records by using an NCBI Nucleotide BLAST search. Phylogenetic trees were constructed in MEGA6 by using maximum likelihood (Tamura *et al.*, 2013).

**Results:** Avian malaria parasites were identified in 45.2% of birds sampled (71/157). Sequence data for these malaria parasites indicated that 97.1% (69/71) were *Haemoproteus* spp. and 2.81% (2/71) were *Plasmodium* spp.; *Leucocytozoon* spp. were not detected. The prevalence of avian malaria parasites was 21.4% in 14 Japanese green pigeons, 42.4% in 33 Japanese wood pigeons, 58.5% in 65 Oriental turtle doves, 33.3% in 3 Grey-capped Emerald doves, 62.5% in 16 Taiwan green pigeons, and 11.5% in 26 rock doves. The phylogenetic tree identified 4 clades (tentatively named A, B, C, and D). Clades A and C had 99% sequence identity with sequences of *Haemoproteus* spp. isolated from the Oriental turtle dove in Japan and the Woodhouse's antpecker (*Parmoptila woodhousei*) in Gabon, Africa, respectively. Clade B had 99% sequence identity with sequences of *H. columbae*. Clade D had 100% sequence identity with sequences of a *Plasmodium* sp. isolated from the common house mosquito (*Culex pipiens pallens*) and from a Greenshank (*Tringa nebularia*) in Japan.

**Discussion:** This study demonstrated the prevalence of avian malaria parasites in 6 species of Columbiformes found in Japan. This is the first report on the prevalence of avian malaria parasites in the Grey-capped Emerald doves and Taiwan green pigeons. The prevalence of avian malaria parasites in Japanese doves and pigeons (45.2%) was much higher than that reported previously for Japanese birds (Murata *et al.*, 2002). It is possible that the detection rate reflects the sensitivity of the molecular biological techniques used. The phylogenetic tree suggests that there are at least 4 lineages of avian malaria parasites infecting Columbiformes in Japan. *Haemoproteus* spp. isolated from the Oriental turtle doves and Japanese wood pigeons caught in Okinawa show 100% sequence identity to clade C. This finding is indicative of host-switching between the different bird species at the genus level, despite the strong host-family specificity in *Haemoproteus*, which was suspected previously. As the *Plasmodium* gene sequence from a captive Japanese green pigeon at a zoo was 100% identical to that found in a common house mosquito at the same zoo, it was assumed that host-vector-parasite interactions in infections by avian malaria parasites have been formed at that location. Heavy mortality has been reported in pigeon nestlings infected with *H. columbae*, which share 99% sequence identity with sequences of clade B (Dey *et al.*, 2010). Therefore, more detailed research on avian malaria parasite infections is needed to develop conservation strategies for the endangered species of the order Columbiformes in Japan such as the Japanese wood pigeon and the Grey-capped Emerald dove.

**Keywords:** Avian malaria parasites, Columbiformes, *Haemoproteus*, molecular phylogeny, *Plasmodium*

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**Helminthes Infections of the Glandular Horned Toad (*Xenophrys major*) in the Tamdao National Park, Vietnam**

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**Abstract:**

The glandular horned toad- *Xenophrys major* (Boulenger, 1908) is widely distributed in Asian countries, including Vietnam. A total of 25 glandular horned toads from Tam Dao national park were examined for helminthes. The prevalence, intensity and abundance were studied. Results showed that the high prevalence of nematode infection in toad (92.6%; 25/27; intensity 1-43 nematode/toad). Seven nematode species were identified, includes *Aplectana mantitoshi*, *Batrachostromylus longispiculus*, *Cosmocerca ornate*, *Rhabdias bufoformis*, *Rhabdias* sp., *Meterakis* sp., and *Heligomosominae* sp.. Among them, *Aplectana* and *Cosmocerca* are the most prevalent genera which found in intestine, followed by *Rhabdias*. There were no trematode, cestode, acanthocephalan infection.

**Key words:** nematode, glandular horned toad, *Xenophrys major*, Vietnam

## **PATHOLOGY**

### **First Discovery of Tufted Puffin (*Fratercula cirrhata*) Infected with *Aspergillus fumigatus* in Korea**

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**Introduction:** The tufted puffin is a relatively abundant medium-sized pelagic seabird in the auk (Alcidae) family found throughout the North Pacific Ocean. It is one of three species of puffin that comprise the *Fratercula* genus and is easily recognized by its thick red bill, and yellow tufts which is most distinctive feature and namesake are the yellow tufts (Latin: cirr) that appear annually on birds of both sexes as the summer reproductive season approaches. This bird from dense breeding colonies during the summer reproductive season from British Columbia, throughout southeastern Alaska and the Aleutian Islands, Kamchaka, the Kuril islands and throughout the Sea of Okhotsk. They are found above 42 degree north latitude. Tufted puffins typically select islands or cliffs that are relatively inaccessible to predators. During the winter feeding season, they spend their time almost exclusively at sea, extending their range throughout the North Pacific and south to Japan and California. There is only one report of this bird preserved in Museum of Nature and human Activity, Hyogo, Japan, which was collected from Dumangan, Gyeongheung-gun, Hamgyeongbukdo on August, 2, 1933. This bird was first discovered in South Korea.

**Materials & Methods:** A bird was rescued on a rice field, Kochang (35°28'38.46"North, 126°40'30.03"East), Chonbuk province on June 30, 2014. It was near lake and river, and located in 15-17 km to reach coast of yellow sea. The discoverer thought it as parrot and kept at home for 1 day. And it was delivered to Chonbuk wildlife rescue center. The bird was intensively cared but died after 3 days and submitted to Animal Diagnostic Center, Chonbuk National University. Routine necropsy was done and tissue sections were stained with hematoxylin-eosin (HE), and examined under light microscope. Bacterial and fungal examination was done by culture and PCR.

**Results:** The bird was identified as female tufted puffin (*Fratercula cirrhata*), which was emaciated (469 gm body weight) and dehydrated. Clinical symptom was tachypnea, but this was recovered after fluid therapy and decreased room temperature. It was fed fish (mallotus villosus), and the feeding response was relatively good. However, X-ray showed enteritis image. On necropsy, body condition was poor and yellowish exudate covered on the some part wall of thorax. The significant gross lesions were greyish-white or green colored fungus covered on the air sac, epicardium and kidney. Some hemorrhagic lesion was observed in the lung. The digestive tracts were empty and multiple and hemorrhagic ulcers were scattered on the mucous of small intestine. Histologically, the lung masses were granulomatous with a central area of necrosis containing heterophils. The wall of air sacs was thickened with necrotizing lesions containing numerous branched fungal hyphae. There are numerous fluke in liver. On Sabraoud dextrose agar, colony spreaded rapidly over the surface. Colony, initially white, became velvety and tinged bluish green, which was identified *Aspergillus fumigatus*. *E. coli* was isolated from liver.

**Discussion:** The tufted puffin was first discovered in S. Korea even first record of a specimen had been collected in a museum, Japan. It is unknown how this bird fled to southwestern part of Korea. Ocean climate varies considerably between years and over decades along the Northern Pacific coast, which can lead wrong migration. Or disease could make some birds lost their migrating pathway. This bird was diagnosed as pneumonia and air sacculitis with *Aspergillus fumigatus*, and bacterial enteritis.

**Keywords:** Tufted puffin, Pneumonia, *Aspergillus fumigatus*, First discovery

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### **Case Report: Hemangiosarcoma in a Ring-necked Pheasant with Reticuloendotheliosis Virus Infection**

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**Abstract:** An old male ring-necked pheasant (*Phasianus colchicus*) showed multiple irregular masses in spleen, liver, heart and body cavity. Histopathologically, the spindle-shaped tumor cells with large nucleus replaced most of normal splenic tissue with some mitosis figures and plenty of myelocytes infiltration. The same tumor cells were also found in other organs. Results of immunohistochemical staining for mammalian Factor VIII and CD 31 marker were negative and Masson's trichrome staining was inconclusive. PCR showed avian leukosis/ sarcoma viruses type A, B, C, D, J and Marek disease virus were negative except for reticuloendotheliosis virus. Collectively, the final diagnosis of this case was hemangiosarcoma which was the first case report in pheasant in Taiwan.

**Keywords:** Hemangiosarcoma, Ring-necked pheasant, Reticuloendotheliosis virus

**Introduction:** Hemangiosarcoma is a type of malignant neoplasms that arises from the lining of blood vessels and usually develops in the spleen, heart, kidney, liver and so on. Oncogenic retroviruses tend to cause a variety of tumors and their target cells for transformation include fibroblastic, hematopoietic and epithelial cells [1]. Reticuloendotheliosis virus (REV) is classified as Gammaretrovirus which consists of a group of pathogenic retroviruses. It is more closely related to mammalian type C retroviruses than to avian leukosis/sarcoma virus (ALV), and is capable to cause chronic lymphomatous diseases and acute reticulum cell neoplasm [2]. Ring-necked pheasant, also known as common pheasant, which is easy to be found in woods, farm, scrubs and wetlands. There are about 30 subspecies in five or six groups with only one distinctive species in Taiwan. In this study, a case sent by the wild animal rescue center was an old male ring-necked pheasant with the chief complaint of weight loss and poor appetite. Multiple tumors were found in the body cavity and organs.

**Materials and methods:** During the necropsy, one reddened irregular mass measuring 4×3×2.5 cm was found in the spleen with hemorrhage and multiple masses of varied size were noted in the liver and heart. Another two masses located next to the crop and under the sternum. All the organs and masses were fixed in phosphate-buffered 10% formalin for 24 hours, and then embedded in paraffin before being sectioned. In addition to the hematoxylin and eosin (H&E) staining, Masson's trichrome staining and immunohistochemical staining for mammalian Factor VIII and CD 31 marker, were also used for discriminating those masses. Polymerase chain reaction (PCR) amplification was also performed with the REV, ALV-A, ALV-B/D, ALV-C, ALV-J and Marek disease virus. Condition of PCR involved an initial denaturation for 3 min at 94°C, and thermal cycling by each cycle profile of PCR in 30 cycles consist of denaturation for 30 s at 95°C, annealing for 30 s at 58°C and extension for 3 min, and final extension for 10 min [3].

**Results:** Histopathologically, the spleen was characterized by poorly demarcated with nonencapsulated hemorrhage throughout the parenchyma and composed different size of blood vessels that lined by neoplastic endothelial cells. The spindle-shaped to polygonal tumor cells with large nucleus replaced most of normal splenic tissue with some mitosis figures and plenty of myelocytes infiltration. The liver was multifocally expanded by solid masses composed of anaplastic cells morphologically identical to those seen in the spleen with unobvious border to normal tissue. The lesion in the heart consisted of nests of anaplastic cells that formed bizarre vessels. In addition, tumor cells could also be found in those masses locating next to the crop and under the sternum. Within the lesion, there were variable proliferation of fibrous tissue and fibroblasts between the stroma. Moreover, aspiration pneumonia and roundworms infection in the esophagus and cecum could be seen. The result of immunohistochemical staining for mammalian Factor VIII and CD

31 marker came out to be negative. Result of Masson's trichrome staining was inconclusive. An REV-specific PCR product of 283 bp could be obtained from the DNA extracted in the masses.

**Discussion:** This is the first case report about hemangiosarcoma in a ring-necked pheasant with reticuloendotheliosis virus infection in Taiwan. The spleen was at least ten times larger than usual and might be the primary neoplasm due to severe lesion. Most tumors have been proved that they are related with avian retrovirus. There were some reports about a variety of avian leukosis viruses, especially the avian leukosis virus type F, which induces hemangiosarcoma or hemangioma in pheasants [4]. In this case, the histopathological diagnosis was hemangiosarcoma and Masson's trichrome staining showed negative result that fibrosarcoma or rhabdomyosarcoma could be ruled out. However, the result of IHC staining appeared to be negative, probably due to the host specificity, which caused failed conjugation of antibodies. The same result could be reported in a golden pheasant (*Chrysolophus pictus*) with hemangiosarcoma [5]. Although the tumor could not be confirmed to be induced by ALV, lymphoproliferative disease virus (LPDV) or REV which usually caused acute reticular cell neoplasia or T-cell/B-cell lymphomas that resemble Marek's disease (MD) instead of hemangiosarcoma, the IHC can still be used for detecting retroviral protein, and in situ hybridization (ISH) can also be used for detecting retroviral RNA/DNA in the neoplastic cells. Direct evidences that the neoplastic cells carry the retroviral gene or protein could be anticipated.

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### Pox Virus Infection in Rufous Turtle Dove (*Streptopelia orientalis*) and Rock Dove (*Columba livia*) in Chonbuk Province in Korea

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**Introduction:** Avipoxvirus infection is a well-known disease of birds reported in more than 200 bird species. The virus belongs to the subfamily *Chordopoxvirinae* of the family *Poxviridae*. There have been two reports for poxvirus infection in a Eurasian jay, and turtle doves in Korea. This is the pox virus infection data in dove from 2009 to 2014 in Jeonbuk Rescue and Conservation Center.

**Materials & Methods:** Twelve doves (ten Rufous turtle doves and two Rock doves) were rescued in different location of Chonbuk province with suspected lesion of Avipoxvirus infection in July and August, 2014. The birds were submitted to Veterinary Diagnostic Center in Chonbuk National University for pathologic examination. Among them, four birds were juvenile and eight birds were adult. Tissue samples were collected, fixed in 10 % buffered formalin and processed routinely with hematoxylin and eosin (H&E) for histopathological evaluation. DNA was extracted from fresh tissue samples and tested by a polymerase chain reaction (PCR) assay using specific primers for 4b core protein gene of avian poxvirus. The prevalence of pox infection was analyzed in rescued dove from Chonbuk wildlife rescue center from 2009 to 2014.

**Results:** Every bird was emaciated and appeared to be depressed. Several cutaneous tumor-like lesions, predominantly on the unfeathered areas of their bodies such as eyes, beaks and feet was observed. Some cases had severe periorbital swelling causing sight impairment. Three of the animals had round, yellowish masses on the hard palate and crop. Histologically, many enlarged epidermal cells exhibited ballooning degeneration and contained round eosinophilic intracytoplasmic Bollinger bodies. Following PCR, poxvirus was detected from all infected animals. In 2009-2014, 317 doves (228 Rufous turtle doves, 89 Rock doves) were rescued in Chonbuk wildlife rescue center and 55 doves (42 Rufous turtle doves, 13 Rock doves) were infected by avian poxvirus. The infection rate in Rufous turtle doves (18.4%, 42/228) was higher than that in Rock doves (14.6%, 13/89). The infections of avian poxvirus in doves are found almost year-round. Infected Rock doves were rescued inside of the city. Rescued site of Rufous turtle doves were focused on the two cities. But these sites were close to parks, suburban hill or mountain, mainly in Jeonju and Iksan, which are the 2 big city in Chonbuk province.

**Discussion:** This study investigated the incidences of avipoxvirus infection in doves in chonbuk province, Korea. Considering high incidences of pox infection, dove may act as important infectious agents to poultry or other avian wildlife.

**Keywords:** poxvirus, Rufous turtle doves, Rock doves, Chonbuk province

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### Ingestion of Marine Debris in a Rough-toothed Dolphin (*Steno bredanensis*)

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**Introduction:** Marine pollution due to plastic debris and other foreign materials has been a major environmental, ecological and economical problem and could be a threat to marine animals all over the world (UNEP, 2009). More than 260 species including sea turtle, seabird and marine mammal are reported to have suffered from entanglement and/or ingestion of marine debris. Even small amounts of debris can have large effects when these things partially or totally block the digestive tract (Laist, 1987). Here we report a case of gastric impaction related to the ingestion of large amounts of marine debris in a rough-toothed dolphin (*Steno bredanensis*).

**Materials & Methods:** An immature female rough-toothed dolphin (*Steno bredanensis*) was stranded on the seashore in Jeju Island and moved in a displaying aquatic facility. The animal was severely emaciated and vomiting was main symptom after feeding. After 5 days of intensive care, the dolphin died and was submitted to the Whale institute for necropsy.

**Results:** Grossly, the dolphin was severely emaciated. There was no evidence of scars or entanglement injuries on the external body and tongue. Necropsy of internal organs was processed and diluted stomach which occupied more than half of abdominal cavity was found. In the stomach of the dolphin, large

amounts of marine debris including plastic bags, thick ropes and other foreign bodies weighting about 3.15kg were found and mucosal ulceration, and necrosis of stomach, pancreas and upper parts of intestine were also noted. Hundreds of parasites were found in the hepatopancreatic duct, which might lead chronic inflammation and weight loss.

**Discussion:** Based on the necropsy of rough-toothed dolphin, gastric impaction due to marine debris was diagnosed as a cause of death. Discarded marine debris has severe impact on marine mammal, and urgent practical managements at national levels and education campaigns for preventing the contamination of marine environment are needed.

**Keywords:** rough-toothed dolphin, marine debris, gastric impaction

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### Histopathologic Study of Cardiomyopathy in Live-Stranded Cetaceans in Taiwan

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**Introduction:** Live-stranded cetaceans occurred very often on the coast of Taiwan every year [1] which prone to sudden death during rescued by organizations that supported by government funding. The purpose of this study is to investigate the cardiovascular histopathologic changes in live-stranded cetaceans that died during rescue.

**Materials & Methods:** Ten live-stranded cetaceans of 4 different species which were alive when discovered but died during further rescue on the southern coasts of Taiwan from 2012 to 2013 were chosen in this study. Professional transportation, medication and rehabilitation had been provided to 10 live-stranded cetaceans by experienced personnel of Marine Biology & Cetacean Research Center of National Cheng Kung University for less than 24 hours to more than 27 days before death. Cardiac muscle samples were collected from both atria and ventricular free walls and the interventricular septum during necropsy which were fixed in 10% neutral-buffered formalin, paraffin embedded, serially sectioned into 5 µm, and stained with haematoxylin and eosin (H&E) and Masson's trichrome techniques.

**Results:** All of the 10 animals showed dilatation and congestion of ventricular cavity on gross examination. Histopathological lesions in the myocardium of cetaceans included congestion, hemorrhages, hypereosinophilic fibers, wavy fibers, perinuclear vacuolization, contraction band necrosis, and focal myocarditis. Masson's trichrome stain revealed focal mild myocardial fibrosis and moderate perivascular-interstitial fibrosis of intramural coronary arteries.

**Discussion:** In this study, the histopathological changes recorded in 10 live-stranded cetaceans showed no obvious differences among species, age, gender, and rescue duration. Myocardial lesions were observed include congestion, hemorrhages, hypereosinophilic fibers, wavy fibers, sarcoplasmic and perinuclear vacuolation and contraction band necrosis. Similar cardiac lesions had also been described in capture myopathy of wildlife [2] and alarm reaction in odontocetes [3]. Cardiovascular lesions were observed perivascular-interstitial fibrosis that showed the perivascular fibrosis of intramural vessels with extensions into the contiguous interstitial space has been referred to as a "reactive fibrosis" [4], accompanied with chronic elevations in the effector hormones of the renin-angiotensin-aldosterone system [5]. The result suggested the cardiovascular lesions in this study had occurred before stranding which may related to long-term stress and were easily leading to tissues ischemic injury. Further studies are required for more expanded sampling and testing of stranded to confirm if cardiomyopathy has a relation to long-term stress due to natural habitat disturbance.

**Keywords:** Cardiomyopathy, Live-stranded, Cetacean

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### Wound Healing of Flipper by Marine Debris in Caged Beluga Whales (*Delphinapterus leucas*)

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**Introduction:** The beluga is an Arctic and sub-Arctic cetacean, which is included IUCN's red list. The effects of marine debris on marine mammals are major problems all over the world. Marine mammal populations are being increasingly impacted by disease and exposure to environmental contaminant, included marine debris. Here we report a case of management of flipper injury related to marine debris in caged beluga whales (*Delphinapterus leucas*). These dolphins belonged to Primosky aquarium, which is being built in Russky Island.

**Materials & Methods:** The beluga whales (*Delphinapterus leucas*) were kept in outdoor cage in Paris bay (43°1'8"N 131°55'12"E.), Russky Island, which was belonged to Primosky Aquarium. There were 2 compartments in cage and 6 beluga (1 ♂, 5 ♀) were kept in each compartment based on age (4 years old and 5-10 years old). Each size of cage was 12 × 18 m and depth was 8 m. On June 2014, 3 younger belugas in one compartment were hurt on the flipper by marine debris, but no any injury was found in older dolphin. Injured belugas were applied amoxicillin and clavulanic acid by 20 mg/kg PO TID, lincomycin by 7 mg/kg PO TID for 15 days, tetracycline ointment TID for 40 days. After treatments, oxytetracycline spray TID was treated for 20 days.

**Results:** Among younger beluga whales, three belugas had flipper injury, which was deep enough to be revealed muscle and phalangeal bone at the anterior part of flipper in the most severe case. The wound size varied from 5- 20 cm. Affected animals were all female. The damages were both right and left, or one side flipper. Immediately, divers investigated water inside of cage. Three sides of cage net were covered with seaweed and some were found in the water, flocks of mussels covered on the nets. The bottom is covered by very thick (more than one meter) layer of silt. Some marine debris, including large metal parts, could make injury in beluga's flippers during swimming. In spite of cleaning seaweed and debris in cage net, new ones grew and found at the cage, which lied on the bottom of the sea, due to limited depth of the place. The wounds were covered inflammatory exudate in the early stage. After 2 month, the granulomatous tissues were formed even the bone was not covered. In the late August, 4 month after injury, the wound size became about 3 cm and bone was covered without any complication.

**Discussion:** The marine debris was speculated the main sources of injury in these belugas during their swimming. Systemic antibiotic treatment prevents generalization and extension of this process. Wound – healing process is similar to that of terrestrial mammals. However, hypertonic environment provides normal wound drainage that allows to keep the wound uncovered. The wounds are recovering successfully.

**Keywords:** beluga, marine debris, wound healing

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***Candida parapsilosis* and *Candida tropicalis* Infection in an Okhotsk Snailfish, *Liparis ochotensis***

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**ABSTRACT**

**Introduction:** Okhotsk snailfish is a ray-finned fishes belongs to family Liparidae. The fish is distributed in Sea of Japan, Sea of Okhotsk, Pacific of Hokkaido islands and northern and eastern Bering Sea. Both *Candida parapsilosis* and *Candida tropicalis* were common in marine environment. They are frequently identified as human pathogens responsible for invasive candidiasis and candidemia, respectively.

**Materials & Methods:** One Okhotsk snailfish was submitted after sudden death from an aquarium in Japan for necropsy and histopathological examination. Molecular diagnosis using seminested PCR assay was performed.

**Results:** Severe necrosis was observed in gill branches. Granulomatous lesion were observed in the gill rays composed of pathogen hyphae, lymphocytes, macrophages and blood cells. The affected primary lamellae were necrosed and exfoliated. The surrounding blood vessels were thrombosed. The lining epithelia of secondary lamellae were hypertrophied. Multifocal granulomas were observed in renal and hepatic tissues. By PAS and Grocott's methenamine silver stains, the microorganisms were long pseudohyphae and blastoconidia that arise singly or in small groups along the pseudohyphae. Immunohistochemistry using anti-Aspergillus, Anti-Rhizomucor and anti- *Candida albicans* antibodies revealed negative staining except for mild positivity for *C. albicans*. seminested -PCR using universal primers for *Candida* sp. and specific primers for *C. albicans*, *C. parapsilosis*, *C. tropicalis* and *C. glabrata*, successfully amplified 100bp and 140bp specific for *C. parapsilosis* and *C. tropicalis*, respectively and failed to amplify *C. albicans* and *C. glabrata*.

**In conclusion,** *C. parapsilosis* and *C. tropicalis* has been recovered from cats and cockatiels after history of eating marine foods, but they have not been recovered from diseased aquatic organisms. This is first report of *C. parapsilosis* and *C. tropicalis* infection in fish species.

***Dirofilaria immitis* Infection of a Farmed Asiatic Black Bear (*Ursus thibetanus japonicus*) in Korea**

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**Introduction:** The genus *Dirofilaria* includes several species that are widely known to cause zoonotic Dirofilariasis. Among these species, *Dirofilaria immitis* infection is an important parasitic disease in many mammals, especially canidae. The filarial species are rarely reported in bear. The bear is not a suitable host for *D. immitis* because adult female worms do not produce microfilariae. In this case, two adult *D. immitis* were recovered from the right cardiac ventricle and pulmonary artery of a bear. *Dirofilaria* infection of an Asiatic black bear is the first case in Korea.

**Materials and Methods:** A 12-year-old male Asiatic Black bear (*Ursus thibetanus japonicus*) was died in a farm, Chonbuk province, Korea. The animal was referred to the Chonbuk Veterinary Diagnostic Center for necropsy. The bear routinely had fed a commercial dog chow. However, the bear was fed on rice bran for 3 days before death and clinical symptom was severe diarrhea. Routine necropsy was performed and muscle was collected for DNA analysis. Total cellular DNA was extracted by using genomic DNA from muscle sample. The cytochrome *b* gene was PCR-amplified. Sequences (1140 bp) of mtDNA cytochrome *b* gene were obtained from this animal. These sequences were compared with the corresponding 31 sequences of four Asiatic black bear subspecies and other species. The parasites were collected around the pulmonary artery preserved in 70% alcohol. *Dirofilaria* spp. was confirmed by polymerase chain reaction (PCR) analysis using species-specific primers for *D. immitis* and *D. ursi*. Also, tissue samples were collected, fixed in neutral-buffered 10% formalin, embedded in paraffin, and stained with H&E.

**Results:** For identification taxonomic status of Asiatic black bear, we analyze cytochrome *b* gene. Maximum likelihood tree with 28 cytochrome *b* haplotypes of bear (21 haplotypes from this study and 7 haplotypes from GenBank) was shown. This Asiatic black bear was identical in their sequence to *Ursus thibetanus japonicus*. The body was emaciated. On gross, blood tinged hydrothorax and pulmonary congestion was observed. Parasites were founded in heart and lung. Two worms were collected and measured 12.0 cm and 19.5 cm long, respectively. There were several ulcers on stomach with various sizes. In the pyloric antrum, long band shaped ulceration was found (4 X 30 cm) and its ulcerated wall was thin to be ready for perforation and its surface was covered with whitish debris with black tinged margin. Mesenteric fat was nearly disappeared, and severe hemorrhagic pancreatitis was observed. Histopathologically, significant stomach ulceration was characterized by extensive loss of epithelium and submucosa. These parasites were demonstrated as *Dirofilaria immitis* by PCR analysis

**Conclusion:** In this case, bear was diagnosed severe stomach ulcer with *dirofilaria immitis*. Although *D. immitis* infection did not seem to the direct cause of death in our case, this infection is generally uncommon in bear and the first report in Korea.

**Keywords:** Asiatic black bear, *Dirofilaria immitis*, Stomach ulcer

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**Clear Cell Variant Mucoepidermoid Carcinoma in a Gray Wolf (*Canis lupus*)**

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**Introduction:** Mucoepidermoid carcinoma (MEC) is the most frequent type of malignant epithelial salivary gland tumor. Microscopically, MEC is composed of three cell types in varying proportions: mucus-producing cells, epidermoid (squamous) cells and undifferentiated intermediate cells. In addition to these essential cell types, clear cells are represented in varying proportions in MEC, and some cases have been reported that clear cells



infrequently predominate over other cell types. Such cases are called clear cell variant MEC. Here we report a rare case of MEC composed of abundant clear cells in a gray wolf (*Canis lupus*).

**Materials & Methods:** A 14-year-old male gray wolf died at the Jeonju zoo and was referred to the Chonbuk Veterinary Diagnostic Center for necropsy. The wolf has a history of severe facial swelling, epistaxis and purulent nasal discharge for approximately 5 months. Clinical and radiological examinations demonstrated an expansile neoplastic mass in the nasal and frontal sinuses, with involvement of the periodontal structures. Tissue samples were collected, fixed in neutral-buffered 10% formalin, embedded in paraffin, and stained with H&E, PAS, PAS-D, and Alcian blue (pH 2.5). In addition, several immunohistochemical stains were performed for differential diagnosis.

**Results:** At necropsy, necrotic purulent discharge was seen in the maxillary gingival. After removing the facial skin, neoplastic mass and extensive necrosis in the nasal turbinate were observed. Microscopic examination revealed that the tumor was principally composed of obvious clear tumor cells. Cytologically, the clear cells had small hyperchromatic nuclei and abundant clear cytoplasm. The clear cells were positive for mucin with PAS, PAS-D reaction and Alcian blue stain. Mucinous materials were seen within the cytoplasm and extravasating into the stroma. Immunohistochemically, myoepithelial antigen such as alpha smooth muscle actins was not expressed in the tumor area. These results were helpful in distinguishing MEC from other types of salivary gland tumors with clear cell morphology.

**Conclusion:** It is known that differential diagnosis of nasal salivary tumor is difficult because salivary tumor has various cellular features. Based on the histopathological features, this wolf is diagnosed with high grade clear cell variant MEC originated from primary salivary gland. To our knowledge, primary tumors of the salivary gland have been reported in cattle, sheep, goats, horses, dogs, and cats. In wildlife animal, however, salivary gland tumors have been scarcely reported. And MEC has not been previously reported in wolf.

**Keywords:** Mucoepidermoid carcinoma, salivary gland, wolf

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### Concurrent Occurrence of Uterus Leiomyoma and Pheochromocytoma in a Bengal Tiger (*Panthera tigris tigris*)

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**Introduction:** Leiomyoma is a benign tumor consisting of smooth muscle neoplastic cells with varying amounts of fibrous connective tissue (Kennedy et al., 1998). Uterine leiomyoma is the most common type, and has been widely reported in dogs, cats, tigers and so on. Pheochromocytoma is a rare tumor originating from the catecholamine secreting chromaffin cells that are derived from the adrenal medulla (Maitra, et al., 2005). Here we report a concurrent occurrence of leiomyoma and pheochromocytoma in a bengal tiger (*Panthera tigris tigris*).

**Materials & Methods:** A 15-year-old female tiger which suffered from paresis of the hind limbs that makes it unable to stand with those legs for 7 months was sacrificed at the Jeonju zoo and was submitted to the Chonbuk Veterinary Diagnostic Center for necropsy. Gross examination was performed and tissue samples of various organs were collected for histopathological examination.

**Results:** At postmortem examinations, a pedunculated, firm, tan-white and solitary tumor (4x7 cm) was attached to the right side of the cervix of uterus. In addition, enlarged adrenal gland was found and pale and well-demarcated mass was located in the middle of the adrenal medulla. No other abnormalities were found. Histological examination revealed that the mass on the uterus was composed of interlacing bundles of proliferating smooth muscle cells mixed with fibrous tissue. The fascicles of cells were elongated and exhibited cigar-shaped nucleus. Mitotic figures were absent, indicating leiomyoma. The neoplastic cells of the adrenal medulla were arranged in nested bundles with a small amount of fibrous connective tissue stroma. Tumor nests were composed of round to oval nucleus cells, and mitotic figures and nuclear pleomorphism were rarely seen, which indicate benign pheochromocytoma.

**Discussion:** Based on the histopathological examination, concurrent occurrence of leiomyoma of uterus and pheochromocytoma was diagnosed. To our knowledge, this is the first necropsy report of concurrence of leiomyoma and pheochromocytoma in a bengal tiger.

**Keywords:** Leiomyoma, Pheochromocytoma, bengal tiger

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### The Incidence of Anthracosis in Zoo, wildlife and Companion Animals of Korea

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**Introduction:** Pulmonary anthracosis is black pigment discoloration by the accumulation of fine particles in the lung parenchyma and is initially regarded as asymptomatic. The presence of anthracosis is an indication that lung has been exposed to air pollutants. To identify the occurrence of dust exposure in common environment of Korea, we conducted an 18-year retrospective study to examine the incidence of anthracosis in carcasses that have been submitted for pathologic examination from 1996 to 2014.

**Materials & Methods:** A total of 255 animals of 55 different species, which lived in common environment of Jeonju City and nearby places in Jeonbuk province, were included in this study. Tissue samples of lung were collected, fixed in 10 % neutral buffered formalin, and processed routinely for histopathology. Tissues sectioned at 6 µm were stained with hematoxylin and eosin (H&E) for histopathological examination. Evaluation of the degree of anthracosis was made according to Schoning et al. (1996) as follows: grade '0' if no particulate material was observed; '1' if a minimal quantity was existed and just sufficient to exhibit its presence; '2' if a moderate quantity was seen, and '3' where either multiple large or many small dust had accumulated.

**Results:** Among investigated animals, 124 cases (48.6%) showed the deposition of black dust particles in the lungs. When the animals were classified by mammal (33 species), avian (20 species) and reptile (2 species) according to the species, avian species (62.3%, 33/53) showed more frequent incidence of anthracosis than mammal (46.4%, 91/196). There was no carbon deposition in reptile species (0/6). The degree of anthracosis was positively associated with age of the animals. In the older group (at least 10 years), almost all of the animals (95%, 19/20) had the deposition of dust in their lung whereas the frequency of anthracosis was low in group of those with 1 year or less (32.3%, 10/31). In correlation analysis between age and the incidence of anthracosis, there was no significance ( $p=0.0961$ ) but positive correlation ( $R=0.9039$ ). In the correlation with age and each grade, the incidence of grade 3 anthracosis showed significant positive correlation ( $p=0.0491$ ,  $R=0.9509$ ). When the subjects were divided into three parts by classification of residence, the highest

frequency of presence of dusty matters in lungs was found in the zoo animals (63.8%, 67/105). The incidence of anthracosis in pet population (43.8%, 32/73) was higher than that of wildlife animals (32.4%, 25/77). In zoo populations, mammal (67.6%, 50/74) and avian species (68%, 17/25) showed similar occurrence of anthracosis. On the other hand, in wildlife subjects, the frequency of carbon deposition in lungs was higher in avian species (57.1%, 16/28) than in mammal (18.4%, 9/49). In pet animals which is mainly composed by dogs and cats, 46.4% (32/69) of dogs contained dusty matters in lungs, and it was not found in the cat (0/4) in collected data. However, no significant correlation was finally observed between the degree of anthracosis and presence of pneumonia.

**Discussion:** This study shows a direct correlation of exposure period to the dust particles with the severity of pulmonary anthracosis. Although the species were diverse in this study, most of the animals that we investigated showed proportional relationship between the amount of dust deposited in lungs and the age. Zoo animals which live in downtown area are usually more easy to be exposed by vehicle exhausted gases or industrial emission than those of wildlife. Our data suggests that the presence of anthracosis might be associated with the animal's living location. Recently, the increasing amount of dust exposure may be correlated with risk factors for chronic respiratory diseases. Although there was no relationship between anthracosis and pulmonary inflammation in this study, further surveillance studies are required to confirm the effects of dust deposition in lungs on respiratory diseases with vast numbers of animals that live in our surrounding environment.

**Keywords:** Anthracosis, Fine dust, Lung, Animals

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#### *Dematophilus congolensis* Infection in Addax (*Addax nasomaculatus*)

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**Abstract:** A case of unusual dermal bacterial infection in a young antelope is reported. There were multiple, hard nodules distributed on the skin and the skin of the affected regions showed variably thickening, rough, and crusted. Microscopically, there is variable thickening of the epidermis due to a down-growing proliferation of the stratum spinosum and granulosum with prominent ballooning degeneration and scattered individual to grouped cell necrosis. There are large numbers of bacterial filaments of varying widths composed of multiple parallel rows of Gram-positive cocci, morphologically resembling *Dematophilus congolensis*. Dermatophilosis is diagnosed in the present case.

**Key Words:** Dermatophilosis, *Dematophilus congolensis*, *Addax nasomaculatus*.

**Introduction:** Dermatophilosis (also known as streptothrichosis) is an exudative, pustular dermatitis that mainly affects cattle, sheep and horses, but also goats, dogs and cats, many wild mammals, reptiles and, occasionally, humans. The principal causative agent is *Dematophilus congolensis* which is a member of the aerobic actinomycete. In the present study, we reported *Dematophilus* spp. infections in an Addax (*Addax nasomaculatus*) and the microorganism is confirmed by hematoxylin and eosin stain, Periodic Acid-Schiff stain and Brown and Brenn tissue gram stain.

**Material and Methods:** One half-year-old *Addax nasomaculatus* was reared in the Taipei Zoo. There are multiple, hard nodules distributed on the skin and the skin of the affected regions showed variably thickening, rough, and crusted. The patient was found dead in the morning of August 25, 2012. The animal was necropsied and representative tissue samples about 1 cm in diameter were fixed in 10% neutral buffered formalin, processed routinely for dehydrated, paraffin embedding, sectioned at 4 µm and stained with hematoxylin and eosin (H&E). Additional sections were also stained by Periodic Acid-Schiff stain and Brown and Brenn tissue gram stain to demonstrate the etiological causes of the lesions.

**Results:** Grossly, there were multiple, 0.5-1 cm, hard nodules distributed on the skin around the mouth, bilateral face, ear, hindlimb, forelimb, and flank, and ventral abdomen and tail, morphologically resembling papillomas. The skin of the affected regions showed variably thickening, rough, and crusted. These crusted nodular growths were frequently entangled with hairs and often easily pilled off. Microscopically, there is variable thickening of the epidermis due to a down-growing proliferation of the stratum spinosum and granulosum with prominent ballooning degeneration and scattered individual to grouped cell necrosis, and moderate to marked parakeratotic and orthokeratotic hyperkeratosis. These affected regions are often crusted, which is composed of degenerate inflammatory cells, keratin, and proteinaceous substance along with the presence of large numbers of bacterial filaments of varying widths composed of multiple parallel rows of Gram-positive cocci, morphologically resembling *Dematophilus congolensis*. By Special staining is positive for Brown and Brenn tissue gram stain and negative for Periodic Acid-Schiff stain.

**Discussion:** In herbivorous animals, *Dematophilus congolensis* grows within the epidermis as filaments from which zoospores are formed; subsequent hyphal growth and formation of a new generation of zoospores occurs only in epidermis. Zoospores resist drying and heating but do not survive long in wet environments outside the lesion. This wetness may have aggravated skin lesions facilitating spread of infection and softening of the skin. Zoospores can be spread between animals by ticks, biting flies, insects, and contaminated pastures. The etiology of the present cases is *Dematophilus congolensis* infection based on the pathological lesions and bacterial morphology found in the hematoxylin and eosin stain and Brown and Brenn tissue gram stain. However, a definitive diagnosis has to be dependent on further examination by polymerase chain reaction (PCR).

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#### Some Pathological Characteristics of Porcine Epidemic Diarrhea (PED) in Wild Boar Breeding Farms

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#### Abstract:

Porcine epidemic diarrhea (PED) is a devastating enteric infectious disease that is characterized by severe enteritis, watery diarrhea, high contagious, out breaking in swine of all ages, high mobility and in particular high mortality in sucking pigs and weaned piglets. The causative agent of this disease, the PED virus (PEDV) is an enveloped, single-strained positive-sense RNA virus. It is a member of the family Coronaviridae and possesses at least 7 open reading frames (ORF1a, ORF1b, ORF2-6) encoding 4 structural proteins and 3 non-structural replicase protein. Gross lesions of porcine epidemic diarrhea showed primarily bloating intestine, thin intestinal, hemorrhage of the intestine. Bloat stomach, containing undigested food. Petechiae of the mesentery lymph nodes and swelling lymph nodes. Microscopic lesions showed peel and cut to pieces villi of the intestine, hemorrhage, inflammatory cell infiltration, degeneration and necrosis in organs such as spleen, lymph nodes, liver, lung and kidney. Porcine epidemic diarrhea virus was detected by RT-PCR. The samples including intestine, mesentery lymph nodes could be used as samples for diagnosis of porcine epidemic diarrhea.

**Key Words:** Porcine epidemic diarrhea, PEDV, pathology, wild boar

**Introduction:** The porcine epidemic diarrhea virus (PEDV), a member of the *Coronaviridae* family, causes acute diarrhea and dehydration in pigs. Although it was first identified in Europe, it has become increasingly problematic in many Asian countries, including Korea, China, Japan, the Philippines, Thailand and Vietnam. The economic impacts of the PEDV are substantial, given that it results in significant morbidity and mortality in neonatal piglets. In Vietnam, PED has just been identified for several years. However, it has occurred in many provinces and caused massive economic losses for farmers. Currently, the knowledge of PED in wild boar breeding farms is rather limited not only with the farmers but also those who do veterinary work in Vietnam.

**Materials & Methods:** 56 wild pigs, from 20 farms, had positive results for PED by examination nRT-PCR were necropsied to observe the macroscopic lesions. The samples (small intestine, cecum, colon, mesenteric lymph node, tonsil, stomach, lung, liver) had been taken for histopathological observation. After 48h fixation in neutral buffered formalin, tissue sections were trimmed, processed, and embedded in paraffin. Four micron thick sections were cut and routinely stained with hematoxylin and eosin.

**Results:** The main clinical symptoms of post-weaning piglets suffering from PED include appetite loss, fever, vomiting, wasting, lying piled especially diarrhea and stool yellow-gray, sticky anus.

Gross lesions mainly in weaned pigs suffering from PED is distended small intestine. Wall of intestine is thin can look inside, hemorrhage, intestinal fluid inside the yellow liquid, piglet. Stomach is distended, haemorrhage and contains undigested food. The lymph nodes, especially the mesenteric lymph nodes show signs of swelling, hemorrhage clearly.

Typical and characteristic of microscopic lesions in PED pigs that: intestinal mucosal blistering, off crushed velvet and gangrene, expressed hyperemia, hemorrhage and infiltration of inflammatory cells in the lower to list opening, in addition to the formation of vacuoles located at the top of villi, the epithelial cells absorb. Epithelial of Gastric was peeling, broken to pieces, the line was destroyed, haemorrhage. The lymph nodes show signs of congestion, hemorrhage, and cystic degeneration lymphocytes, lymph reduce demand. There is also degeneration, hemorrhagic necrosis in some other organs such as the liver, spleen, kidneys, lungs.

**Discussion:** In Vietnam, outbreaks of acute gastroenteritis in wild piglets with clinical signs as yellow-watery diarrhea and lesions as distended stomach, thin small intestinal wall are often diagnosed toward TGE or FED by swine practitioners and producers. So, we think that exact diagnosis of causal agents is a basic prerequisite for application of immunoprophylactic therapies more effective in disease treatment and prevention.

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#### Polycystic Kidney Disease in the Adult Female Pygmy Hippopotamus (*Choeropsis liberiensis*)

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**Introduction:** Polycystic kidney disease (PKD) is characterized by multiple cysts within the renal parenchyma and is an important contributor to renal and systemic morbidity. Although PKD is considered to be heritable in humans, dogs, and cats, the exact mechanisms of PKD remain unclear. PKD has been frequently reported in a variety of domestic, nondomestic, and wildlife species. However, a few cases of PKD have been described in captive pygmy hippopotamuses.

**Materials & Methods:** A 33-year-old, 198-kg female pygmy hippopotamus (International Studbook No. 568) was found dead on 15 January 2013 in Seoul Zoo. The female hippopotamus was born in the Tierpark Berlin, Germany (International Studbook No. 568), on 22 May 1980. At the age of 4 years, in May 1984, it was transferred to Seoul Zoo. A necropsy was performed at the Animal Health Center of the zoo to identify the cause of sudden death of the adult female pygmy hippopotamus. Tissue samples of the lesions were fixed in 10% neutral buffered formalin and processed routinely. Tissue sections were stained with hematoxylin and eosin for light microscopy examination.

**Results:** On necropsy, one kidney was slightly enlarged. The lower part of the other kidney contained a large cyst filled with light yellow-colored watery fluid without renal parenchymal tissue. Both kidneys had numerous variably sized fluid-filled cysts of 2 to 20 mm in diameter. A considerable portion of the renal cortex and medulla was replaced by cysts. On microscopic inspection of the kidney, the cysts were lined with low cuboidal to flat epithelial cells. The diagnosis of PKD was confirmed by gross findings and histopathological examination. Blood-stained peritoneal fluid was observed upon opening the abdominal cavity. Widespread hematomas and congestion were present in the mesentery of the intestine.

**Discussion:** Various PKDs have been described in humans and many domestic animal species including lambs, rabbits and cats. In the present case, both kidneys exhibited numerous variably sized fluid-filled cysts 2 to 20 mm in diameter. One kidney was almost half the size of the other. The cause of these size discrepancy, whether a congenital defect or secondary to PKD, is uncertain. Although the natural course of PKD is not well characterized, it seems to be a multifactorial disease with a complex pathogenesis. Additionally, there is some indication that in genetically susceptible individuals, environmental factors may trigger the initiation and perhaps the progression of PKD. This report is the first case of PKD in a pygmy hippopotamus in Korea, and further study is needed to elucidate the pattern of occurrence of PKD in pygmy hippopotamuses.

**Keywords:** polycystic kidney disease, pygmy hippopotamus, *Choeropsis liberiensis*

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#### Necropsy Findings of Three Wild Indian Crested Porcupines (*Hystrix indica*) Presented to the Veterinary Teaching Hospital (VTH), Sri Lanka

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#### Abstract

Information about diseases affecting Indian Crested Porcupine (*Hystrix indica*) is limited. Necropsy findings of three wild Indian crested porcupines presented to the VTH, University of Peradeniya, Sri Lanka are described in this article. In all three animals main pathological lesions were observed in the cardio-respiratory system. Lesions related to septicemia were evident in two animals and the responsible microorganisms were isolated. Investigation

of morbidity or mortality events of infectious diseases in wild animals give prior knowledge and understanding about such events and current findings will be important to improve health and welfare of the wild and captive crested porcupines.

**Key words:** Indian Crested Porcupine (*Hystrix indica*), wild mammal, cardio-respiratory

### Introduction

Indian Crested Porcupine (*Hystrix indica*) is a common nocturnal mammal with a wide distribution in Southern Asia and Middle East. In Sri Lanka, they are found throughout the lowlands to the highlands, but not commonly found in higher elevations. They are well adapted to urban habitat and are frequent visitors to the home gardens. Porcupines feed on variety of plant matter such as tender leaves, fruits and roots. Because of their adaption to various habitats and the feeding habits, they have become one of the main agricultural pests in many areas of the country. They are one of the small wild mammal species frequently presented to the VTH, University of Peradeniya, Sri Lanka mainly being attacked by dogs or being caught in snares. Currently, little is known about the various diseases affecting the porcupines and published literature is limited<sup>1</sup>. This paper describes the necropsy findings of three porcupines presented to the VTH.

### Case descriptions

**Case 1:** An adult, male porcupine was presented after being attacked by a dog with multiple skin lacerations and deep ulcerated wounds localized to the back of the body. In addition a yellowish, frothy nasal discharge was evident from both nostrils. The condition was diagnosed as a respiratory tract infection and despite the treatments provided (Intravenous fluids, long acting antibiotics and analgesics) the animal died on the following day.

Necropsy performed soon after revealed severe diffuse pulmonary oedema and congestion with severe bronchopneumonia. Mild serosanguineous peritoneal, pleural and pericardial effusions and generalized hyperemia of all organ systems suggestive of septicemia were also evident. Histopathology revealed, mixed inflammatory cell infiltration (predominantly composed of macrophages and neutrophils) in alveolar and bronchiolar regions. Severe diffuse cortical tubular epithelial cell degeneration and necrosis was evident on both kidneys. Large number of white, round, slender worms which were later characterized as *Trichuris spp* were present in semi-solid small intestinal contents.

A pure culture of *Bacillus brevis*, a gram-positive, aerobic, spore-forming bacillus was isolated from the heart blood. This bacterium is a non-pathogenic organism commonly found in soil, air, water, and decaying matter. However it becomes an opportunistic pathogen in diseased or immune-compromised animals.

**Case 2:** An adult female porcupine presented with signs of mild respiratory distress and reduced movements died on the following day of hospitalization. A detailed postmortem examination was performed immediately after the death.

No significant lesions except a frothy blood stained nasal discharge were evident on external examination. Marked sub cutaneous oedema was evident on primary incisions. Furthermore serosanguinous pleural and peritoneal effusions were evident. Gross and histopathological cardiovascular pathology revealed severe, diffuse fibrino-suppurative pericarditis, chronic lympho-histiocytic endocarditis and mild mitral endocardiosis. Pulmonary pathology included severe diffuse pulmonary oedema and severe multifocal pneumonia. Multifocal echymotic haemorrhages were observed in the peri-renal fat over the left kidney. Mucosa of the urinary bladder was moderately and diffusely hyperaemic indicating mild acute urocystitis. In addition severe multifocal suppurative encephalitis was also evident. Gross and histopathological findings all together were suggestive of death due to cardiovascular failure.

**Case 3:** An adult male porcupine presented with dyspnea, cardiac arrhythmias and purulent eye discharges died on the following day. Necropsy performed few hours later revealed multi-focal pulmonary emphysema, severe diffuse pulmonary congestion and oedema, locally extensive hemorrhages on the caudal lung lobes and severe diffuse tracheitis. Moderate to severe diffuse hyperemia was evident on all other organ systems. In addition cardiovascular pathology revealed right ventricular enlargement and mild endocardiosis. A pure culture of *Proteus morganii* was isolated from a heart blood swab.

### Discussion

According to authors knowledge necropsy reports related to cardiovascular pathology in Indian crested porcupines have not been documented previously. Furthermore reports on respiratory pathology on the same species also limited. In addition two of the cases described here include septicemic conditions caused by two bacterial organisms (*Bacillus brevis* and *Proteus morganii*) considered to be non-pathogenic most often. Furthermore *Proteus morganii* is reported as a causative organism often encountered in postoperative and other nosocomial infections such as urinary tract infections in humans suggesting a zoonotic potential of the particular organism.

It is important to detect and document the diseases affecting Indian crested porcupines since they frequently visit the home gardens and share the same environment with human domestic animals.

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## PRIMATE

### Biomedical Evaluation and Disease Prevalence of Three Sympatric Lemur Species in Taipei Zoo

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#### Abstract:

Medical evaluations were performed on captive black-and-white ruffed lemurs (*Varecia variegata*), brown lemurs (*Pteropus fulvus*), and ringed-tail lemurs (*Lemur catta*) at Taipei Zoo. Five clinically healthy lemurs were selected from each species based on medical record review and gross appearance for hematological and serum chemistry evaluations. Blood samples were collected for complete blood cell count, differential white blood cell count, serum biochemical profile, serum iron, serum ferritin, total iron binding capacity (TIBC), total thyroxine (T4), triiodothyronine (T3), and toxoplasmosis serology. Based on the laboratory findings, the prevalence of the 3 commonly seen diseases in captive lemur species such as toxoplasmosis, goiter and hemosiderosis was also investigated in this study.

**Keywords:** black-and-white ruffed lemur, *Varecia variegata*, brown lemur, *Pteropus fulvus*, ringed-tail lemur, *Lemur catta*, blood cell count, serum biochemistry

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C WOOD, SG FANG, A. HUNT, W.K. STREICH, M. CLAUS. Increased Iron absorption in lemurs: Quantitative screening and assessment of dietary prevention, *American Journal of Primatology* 61:101-110(2003)

### Age-related Changes on Hematological, Serum Biochemistry and Blood Gas Parameters in Cynomolgus Monkeys (*Macaca fascicularis*)

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**Abstract:** We have maintained the Aging Farm of cynomolgus monkeys, which was introduced in ASZWM 2012 (Bangkok, Thailand), in Tsukuba Primate Research Center (Ibaraki, Japan). As a part of its characterization, we analyzed somatometric measurements, hematologic parameters, serum biochemistry and blood gas for the aged monkeys and compared with the values of the young monkeys in the facility. Significant differences between old and young animals were found in several parameters of hematologic and biochemistry while little difference were revealed in blood gas values.

**Introduction:** Recently, the use of nonhuman primates (NHPs) to investigate age-related changes has increased. However, few knowledge about aged NHPs are available because of its maintenance difficulty and varied characteristics. Our Aging Farm of cynomolgus monkeys could be an ideal model for aging biomedical research.

**Materials & Methods:** Fifty five healthy cynomolgus monkeys including 9 young monkeys (less than 10 years old), 13 matured monkeys (10 to 20 years old), 21 middle aged monkeys (20 to 30 years old) and 12 elderly monkeys (more than 30 years old) were used in this study. They were bred in the Tsukuba Primate Research Center (Ibaraki, Japan). Their body weight, hematology, serum biochemistry, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and blood gas under anesthesia were examined.

**Results:** The hematologic analysis revealed that age-related differences occurred in WBC count, RBC count, hemoglobin and Hematocrit. Those parameters were increased from young to older aged monkeys. Serum albumin, glucose and ANP were significantly higher in the old monkeys. The aged animals showed no sex-related differences while the young animals showed some sexual distinctions. BNP showed a decreasing tendency with age. Blood gas showed little difference between aged and young monkeys.

**Discussion:** Increasing serum albumin, Glucose, potassium and BE value in aged animals were supposed renal failure with aging in cynomolgus monkeys. It could be significant over 20 years old. Unexpectedly blood gas was appeared no significant difference between the old and the young animals, although, it may be compensated with the function of respiratory system for depression of the metabolism function.

**Key Words:** Cynomolgus monkey, aging, blood

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### Enhanced magnetic resonance imaging of Spontaneous Occurring Hepatocellular Neoplasia in Cynomolgus Monkey (*Macaca Fascicularis*)

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**Introduction:** Spontaneous neoplasia in nonhuman primates had been reported extremely uncommon. However with advancing age in the population, there has been increased the number of reported tumors in nonhuman primates. Just all the same, primary hepatic tumors still appear to be similarly infrequent occurrences in nonhuman primates, with the notable exception of prosimians with spontaneous neoplasms in liver. In human, radiography or ultrasonography could not be achieved to assess and to diagnose clearly the differentiation of the organs or their abnormal structures where include masses. Superparamagnetic iron oxide enhanced-MRI (SPIO-MRI) tends to be used practically to identify the tumors and to pinpoint their size, location and the border of mass and normal tissues in the liver noninvasively.

**Materials & methods:** A 13-y-old female cynomolgus monkey in Tsukuba Primate Center exhibited signs of clinical illness such as anorexia, with no serological abnormality of liver enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST). After fluid administration, the condition had been in remission a few months under observation. Laboratory analysis of serum parameters after two weeks of recovery showed the high rate of creatinine (CRE), alkaline phosphatase (ALP), lactate dehydrogenase (LDH). In addition, we could confirm the presence of firm palpable mass and checked it with ultrasonography, radiology (both of these data were not shown) and SPIO-MRI with 3T MR scanner. After we got pre-image with MRI, we administered Ferucarbotran (the final iron concentration was 0.45 mg Fe/kg), which is one of SPIO, as contrast agent. Then we got post-image to assess the hepatocellular neoplasia in her liver noninvasively.

**Results:** According to T2-weighted images, the contrast of masses and other normal liver tissues were clearly described. And T1-weighted images could not show any contrast with Ferucarbotran than T2-weighted images.

**Discussion:** T2-weighted images could show the border of masses and liver clearly, and that were same images as pictures at exploratory laparotomy. On the other hand, T1-weighted images couldn't show any useful contrast with Ferucarbotran. T2-weighted images by SPIO-MRI are therefore practicable for the diagnosis of hepatocellular neoplasia in nonhuman primates.

**Keywords:** Cynomolgus monkey, hepatocellular neoplasia, SPIO-MRI, Ferucarbotran

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### Nearest Neighbour Changes in Captive Infant Francois' Langurs (*Trachypithecus francoisi*)

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**Introduction:** Nearest neighbor (NN) have been studied in various primate species, such as chimpanzees (*Pan troglodytes*), bonobos (*P. paniscus*), western lowland gorilla (*Gorilla gorilla gorilla*). However, studies on nearest neighbors in colobine monkeys are quite limited. Furthermore, there is no research on the relations between an infant and other individuals, including its mother, in the social group. According to McKenna (1979), high levels of



infant caregiving behavior by non-mother females (allomothering behavior) can be observed in colobine monkeys, even on the day of birth. To understand infant social development, it is important to ascertain social relations not only between an infant and its mother, but also between the infant and other group members. In this report, we present NN observations among two infants and other individuals in a captive group of Francois' langurs (*Trachypitecus francoisi*) belonging to the Colobinae subfamily.

**Materials & Methods:** We observed a captive social group consisting of two mother-infant pairs (temporarily termed mother A and infant A [birth date: November 13, 2013], and mother B and infant B [birth date: December 15, 2013]) and one father (the father of both infants) of Francois' langurs at ZOORASIA Yokohama Zoological Gardens, Japan. Mother A and mother B were siblings. We collected NN data, and distance to NN, for two infants over 7 months following the birth. Observations were conducted for about 3 hours per day, once a week. A one-minute interval scan sampling method was used for recording. NN distances were categorized as *ventral*: in ventral contact with the other individual; *contact*: most of the infant's weight is supported by the ground or vegetation, but the infant's body is touching the other individual's body; *arm reach*: infant staying within arm's reach of the other individual, except for *contact* (<60 cm); and *out of reach*: infant staying beyond the mother's reach (>60 cm).

**Results:** In the case of infant A, mother B was recorded as the most frequent NN at 20 days of age (mother A = 17.2%, mother B = 82.8%). At age one month, mother A spent a higher proportion of time as NN than mother B (mother A = 89.0%, mother B = 11.4%). At 2 months, infant B was recorded as the NN of infant A for the first time (mother A = 88.5%, mother B = 9.2%, infant B = 2.3%). At 3 months, the proportions for infant A's NNs were similar to that of NNs at 2 months. At 4 months, the father was recorded as infant A's NN for the first time (mother A = 85.2%, mother B = 8.1%, father = 2.7%, infant B = 4.1%). At 5 months, infant B was recorded as infant A's NN more frequently than at 4 months (mother A = 78.6%, mother B = 4.6%, father = 3.4%, infant B = 13.3%). At 6 months, the proportions of infant A's NNs were similar to that of the NNs at 5 months. However, infant B spent 100% of time with mother B within the 0 months of age. At one month, mother A and infant A were recorded as infant B's NN for the first time (mother A = 0.5%, mother B = 97.1%, infant A = 2.3%). At 2 months, the proportions of infant B's NNs were similar to that of NNs at one month. At 3 months, the father was recorded as infant B's NN for the first time (mother A = 2.1%, mother B = 93.9%, father = 0.3%, infant A = 3.5%). At 4 months, the time that infant A spent with infant B had increased since 3 months (mother A = 1.7%, mother B = 86.6%, father = 1.0%, infant A = 10.6%). At 5 and 6 months, the proportions recorded for the NNs of infant B were similar to those at 4 months of age. The proportions of infant A's NN distances were *ventral*: 98.9%, *contact*: 0%, *arm reach*: 0%, and *out of reach*: 1.1% at 20 days of age. After age one month, the *ventral* tendency decreased, but *contact*, *arm reach*, and *out of reach* tendencies increased. The proportions of infant A's NN distances at 6 months were *ventral*: 49.4%, *contact*: 20.8%, *arm reach*: 14.7%, and *out of reach*: 15.1%. In the case of infant B, the proportions of the NN distances were *ventral*: 99.6%, *contact*: 0.4%, *arm reach*: 0%, and *out of reach*: 0% at the 0 months of age. After the first month, the *ventral* tendency decreased, but *contact*, *arm reach*, and *out of reach* tendencies increased. The NN distances of infant A at 6 months were *ventral*: 50.1%, *contact*: 17.0%, *arm reach*: 19.5%, and *out of reach*: 13.3%.

**Discussion:** At 20 days of age, infant A spent 82.8% of time with mother B, and its most frequent NN distance was *ventral* (98.9%). One previous study indicated that chimpanzee infants spent more than 90% of the time with their mothers at ventral distances within 0 months of age. In comparison, we observed that infant A spent much more time (82.8%) with mother B. In earlier studies on colobines, allomothering behavior was observed on the first day of life, e.g., for the common gray langur (*Presbytis entellus*), the Douc langur (*Pygathrix nemaeus*), the black and white colobus (*Colobus polykomus*), the proboscis monkey (*Nasalis larvatus*), and the dusky leaf monkey (*Trachypitecus obscurus*). Furthermore, according to Krishnamurthy (1992), allomothering behavior was observed among captive Francois' langurs during the first five days of birth. In light of these reports, the results of the observation that infant A spent 82.8% of time with mother B in 0 months of age, suggests that allomothering had occurred early stage of growth on an equality with the other colobines. After the birth of infant A, the ratio of caregiving by mother A for infant B had decreased remarkably. Moreover, there were no records showing mother A as infant B's NN at 0 month of age. These results suggest that allomothering behavior occurs at a high rate if other females in the group do not have babies. Our results show infant development in Francois' langur social groups is affected by the presence of non-mother females and/or other infants. In addition, our observations suggest that allomothering behavior could occur at an early stage of infant growth if there are some females who have a high rate of consanguinity. To clarify the relationships between allomothering behavior and kinship in Francois' langur, research is needed on social groups containing mothers with no kinship links.

**Keywords:** Colobinae, Francois' langur, infant, nearest neighbor (NN)

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#### Pyothorax with *Prevotella loescheii* in a Common Marmoset (*Callithrix jacchus*)

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**Introduction:** *Prevotella spp.* are anaerobe Gram-negative bacteria and known as human oral flora and opportunistic pathogen. It has been reported that they were isolated from abscesses, pulmonary infections, sinusitis, and ear infections. Predisposing factors for *Prevotella spp.* infections are immune suppression, metabolic disease, neoplasia, previous surgery and dental procedures in primates.

This case report describes respiratory infection due to *Prevotella loescheii* in a common marmoset.

**Materials & Methods:** An expired 4-year-old male common marmoset was presented to necropsy with weight loss, anorexia and fever. A gradual weight loss was noticed 2 weeks ago. Hematology and serum chemistry analysis were done antemortem, and radiograph and computed-tomography were investigated postmortem. Bacterial isolation and molecular identification were done from the purulent pleural effusion which was collected in a necropsy.

**Results:** Hematologically, there was regenerative anemia. Chemistry profile showed elevated liver enzyme and total bilirubin, and low albumin and BUN. Thoracic radiograph and CT scan revealed presence of pleural effusion. Grossly, there were cyst in lung and purulent effusion in thoracic cavity. Neutrophilic inflammation and bacterial infection were found on cytology of lung and pyothorax. Following bacterial isolation for pleural effusion, *Prevotella loescheii* was identified with molecular method.

**Discussion:** Pathogenic agents in respiratory tract of marmosets are Parainfluenza virus, measles virus, *Klebsiella pneumoniae* and *Bordetella bronchiseptica*. *Prevotella loescheii* is a member of oral commensal and pathogen of periodontal infection in human and marmosets. However, it can be an opportunistic pathogen for respiratory tract causing death in common marmosets.

**Keywords:** Marmoset, *Prevotella loescheii*, pleuritis, pneumonia

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**Patterns of Reproductive Hormone Excretion during Pregnancy in a Chimpanzee (*Pantroglodytes*) and Orangutan (*Pongo pygmaeus*)**  
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**Introduction:** The secretory patterns of reproductive hormones in wild- and zoo- animals had been investigated by the Species Conservation Projects of Endangered Wild Animals in Seoul Zoo, Korea since 2006. The reproductive hormonal patterns of Chimpanzee and Orangutan are quite similar to that of Human: Long pregnant period, amenorrhea in lactation, cyclic menstrual changes etc. The diagnostic methods to determine the pregnancy of human can be applied to primates. However, direct diagnostic methods (blood collection, ultrasound, X-ray) are seriously difficult and dangerous to zoo staffs and zoo animals. The monitoring of fecal and urine hormone is suggested to invasive simple method. Therefore we tried to study reproductive hormone by using invasive method. The research can demonstrate the reproductive hormones of pregnancy period and provide new scientific information.

**Materials & Methods:** The animals used in this study were young mature Chimpanzee and Orangutan in Seoul zoo. Fecal and urine samples were collected twice weekly from mid time to delivery. Collecting fecal samples were lyophilized for 48h. The reproductive hormone metabolites were extracted from dried feces (0.2g) by mixing diethyl ether (10ml) and PBS (2ml). The metabolites of fecal and urine hormones were assayed by time-resolved fluoro-immunoassay (TR-FIA).

**Results:** Measurement of fecal steroids was used to monitor pregnancy status in Chimpanzee and Orangutan. Progesterone levels were elevated significantly in the fecal samples of pregnant periods than those of non-pregnant periods: It increased rapidly from mid term to last term, but decreased to base line until delivery time. The pattern of fecal estradiol was different from two species. In Chimpanzee, it was increased with progesterone. In case of Orangutan, it was baseline until delivery date.

**Discussion:** Urinary secretion patterns of estradiol and progesterone in Chimpanzees and Orangutans have been reported (Czekala et al., 1983). However the collection methods of urine and blood samples have many disadvantages: not easy, invasive, laborious, quite dangerous etc. Nowadays, the metabolites of sex steroid hormones can be measured in feces of wild animals (Schwarzenberger, 2007). We had established the non-invasive methods using the fecal samples to monitor the reproductive patterns of zoo animals. The patterns of fecal estradiol and progesterone levels were different from two species. In Chimpanzee, estradiol was increased with progesterone. In case of pregnant Orangutan, the pattern of fecal estradiol was different from that of previously urinary pattern. This study suggests that the measuring concentration of fecal progesterone in both species is the efficient method to determine pregnancy.

**Keywords:** Estradiol, Feces, Progesterone, TR-FIA

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**Urinary Sex Steroid Hormones and CG during Pregnancy  
in the Borneo Orangutan (*Pongo pygmaeus*)**

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**INTRODUCTION**

Hormonal levels change dramatically in pregnant women. Levels of progesterone and estrogens rise continually throughout pregnancy. In general, because human *chorionic gonadotropin* (hCG) is produced only from placenta, detection of CG in urine is used for pregnancy diagnosis. Estriol is one of the three main estrogens and increases the production in pregnancy. In late pregnancy, it had been reported that estriol is the major estrogenic component in urine by high-pressure liquid chromatography (HPCL) in orangutans. However, changes in urinary estriol levels have not been reported in orangutans.

The present research deals with the measurement of urinary chronic gonadotropin, estriol glucuronide, and other sex steroid hormones and make it clear that change reproductive endocrine in pregnant orangutan.

**METHODS**

One female orangutan, named Rorrie (born on 21 June 1979) was used in this study. This animal was housed in Kushiro City Zoo. We collected urinary samples from November 7, 2009 through March 4, 2010 (parturition on March 1, 2010) and from June 12, 2013 through August 21, 2013 (parturition on July 15, 2014). We analyzed Estrone conjugates ( $E_1C$ ), Estriol glucuronide ( $E_3G$ ), Pregnanediol glucuronide (PdG) and chronic gonadotropin (CG) using enzyme immunoassays.

**RESULTS and DISCUSSION**

We analyzed Estrone conjugates ( $E_1C$ ), Estriol glucuronide ( $E_3G$ ), Pregnanediol glucuronide (PdG) and chronic gonadotropin (CG) in urine by ELISA. And we showed changes in levels of urinary  $E_1C$ ,  $E_3G$ , PdG, and CG throughout pregnancy.

The results showed that urinary  $E_1C$ ,  $E_3G$ , PdG, and CG levels were increased during early pregnancy (samples of 2013 year). During mid- and late pregnancy (samples of 2009 to 2010 year), concentrations of urinary  $E_1C$ ,  $E_3G$  and PdG levels maintained high levels. On the other hand, urinary CG levels did not change throughout our experimental period. It is considered that CG level may reach a peak value between 9-13 week of pregnancy and then it may decline to a nadir. Finally, Levels of all hormones declined to non-pregnant range within a few days after the parturition. These results suggest that these hormones are useful to monitoring pregnancy in orangutans.

**Cutaneous Undifferentiated Sarcoma in a Sumatran Orangutan (*Pongo abelii*) in Reintroduction Forest of Bukit Tigapuluh Landscape**

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**Abstract:** A 4-years-old female orangutan presented with a skin lesion on her middle back. During two weeks, the lesion became larger up to 2 cm in diameter. The lump bleeds and developed a crust. It was surgically excised and histopathological examination was done for the resected specimen. There is emarginated and non-capsulated tumour expands dermis layer which covered by ulcerative epidermis. The healing progress was good and still no chance of relapse. Immunohistochemistry staining using vimentin may be utilized to confirm cutaneous sarcoma.

**Key words:** sarcoma, cutaneous, undifferentiated, Sumatran orangutan

**Introduction:** Sarcoma is malignant tumour of soft tissue that constitute a highly heterogeneous group of neoplasms histologically classified according to the mature tissue they resemble (Enzinger and Weiss, 2001; Fleury and Sanches, 2006).

**Materials and Methods:** A 4-years-old female Sumatran orangutan (*Pongo abelii*) presented with The resected specimen was fixed in 10% neutral buffered formalin (NBF) and embedded in paraffin. Five-micrometer-thick section was stained with haematoxylin-eosin (HE).

**Results:** Histopathological examination of the excised lump showed that there is emarginated and non-capsulated tumour expands dermis layer which covered by ulcerative epidermis. Tumor cells population contained interlacing woven and storiform patterns which constructed by smooth fibrovascular stroma. Those tumor cells are fusiform with minimize eosinophilia cytoplasm, rounded nucleus, high ratio of nucleus within cytoplasm, smooth granular chromatin and in every nucleus is contained 1-2 nucleolus. There is multifocal microhaemorrhage, dotted necrosis and infiltration of mononuclear inflammatory cells on tumour margin area.

**Discussion:** Malignant tumour or sarcoma, on the other hand, is locally aggressive and capable of invasive and destructive growth, local recurrence and metastases (Enzinger and Weiss, 2001; Guillen and Cockerel, 2001). Sarcoma is usually manifested by deep lesion, but it may affect the skin and subcutaneous tissue, presenting as a superficial lesion or subcutaneous nodule. Excisional biopsy was the only choice to prevent the tumour became larger, since the reintroduction forest was very isolated area with minimum medical facility.

**Conclusion:** Cutaneous sarcoma is rare tumour. Immunohistochemistry staining using vimentin may be utilized to confirm cutaneous sarcoma or rule out other spindle-cell proliferation.

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#### Non- invasive Assessment of Stress and Parasite of Orangutans (*Pongo spp*) in Captivity

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**Abstract:** Non-invasive methods based on urinary and faecal hormone measurements is the most precise of the indirect methods of monitoring physiology of stress. The method has been used to monitor stress in orangutan by measuring faecal glucocorticoid metabolites (FGM). Faecal samples were collected from 16 adult individuals orangutans comprised of 13 Bornean and 3 Sumatran Orangutan at Ragunan Zoo, Jakarta. Faecal hormone analysis was measured using EIA 11 $\beta$ -hydroxyetiocholanolone. Laboratory validation showed that standard curve of 11 $\beta$ -hydroxyetiocholanolone was parallel to the curve of sample dilution. Stress levels among individual showed a significant differences (P <0.05), among the enclosure also demonstrate statistically significant differences (P <0.05). On the examination of parasitic worm the highest prevalence of worm infection is the egg of 100 % Ascarids, 94 % for Strongyloides, 100 % for Strongyloids and 19 % for Trichurids. Some of the enclosure condition are correlated to the level of worm infection and stress levels such as exhibition enclosure size, lighting conditions, and the type of cage flooring. But in general these parameters only showed weak correlation. We found no correlation between the number of parasite eggs and stress levels observed in orangutans.

**Key Words:** Orangutan, Stress, Parasite, Captivity

**Introduction:** Being placed in captivity may cause stress in animals that may lead to immunosuppression which can magnifies the prevalence of some diseases and increasing the susceptibility to infections or expression of latent diseases. In the present study we investigated the level of the stress hormone and prevalence of parasitism and correlated those parameters with the enclosure condition to monitor the wellbeing of orangutans in captivity.

**Materials and methods:** Faecal samples was collected from 16 adult individuals orangutans comprised of 13 Bornean (8 Females ; 5 Males) and 3 Sumatran (1Females ; 2 Males). FGCM was measured using enzyme immune assay (EIA) against cortisol metabolite, 11 $\beta$ -hydroxyetiocholanolone (3 $\alpha$ ,11 $\beta$ -dihydroxy-CM). Helminth eggs were recovered using qualitative and quantitative methods.

**Results:** Biological validation was determined through the increase of FGM when orangutan was affected by stressor such as movement, release, anesthesia, and surgery. There was two days time lag of FGM excreted into faeces, FGM concentration was increased two days after surgery and after transfer to new facility. Stress levels among individual showed a significant differences (P <0.05), among the enclosure also demonstrate statistically significant differences (P <0.05). On the examination of parasitic worm the highest prevalence of worm infection is the egg of 100 % Ascarids, 94 % for Strongyloides, 100 % for Strongyloids and 19 % for Trichurids. Some of the enclosure condition are correlated to the level of worm infection and stress levels such as exhibition enclosure size, lighting conditions, and the type of cage flooring. But in general these parameters only showed weak correlation. We found no correlation between the number of parasite eggs and stress levels observed in orangutans.

**Discussion:** According to the information from keeper the significantly higher stress levels was caused by temporary placement of Sumatran orangutan in Bornean orangutan block. This may explain the high level of stress that might be caused by behavioral stress. Parasitic worm infection is not only influenced by several parameters that have been analyzed but also the immune factors and the treatment of each orangutan by anthelmintic during the sampling time. In this study we didn't find any correlation between levels of FGCM and parasite burden, even on some individual such showed heavily infestations of parasitic worm, it only showed and average levels of FGCM. But due to the limitation of this study we might be missed different parasite such as protozoa. Higher FGCM value of some individuals seems to be noises caused by behavioral stress that might be acting temporal without impairing immune system.

**Conclusions:** Non - invasive techniques we use has successfully monitor the level of parasitic worm infections and stress levels in BLUD Taman Margasatwa Ragunan. Some of the enclosure condition are correlated to the level of worm infection and stress levels such as exhibition enclosure size, lighting conditions, and the type of floor cage. But in general these parameters only showed weak correlation. Some individuals showed a higher stress levels than other individuals. But this might be a temporary stress caused by behavioral stress (e.g placed with different species or other adults male). In general we found no correlation between parasitic worm infections with the level of stress on orangutans in TMR. So we can't concluded yet that the suitability of parasitism as a proxy for immune function in this study. In the future more comprehensive research needs to be done with the addition of parasitic protozoan and behavioral observation.

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## OTHERS

### Center Karst and Plateau of Vietnam as a Center of Biodiversity of Small Mammals

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**Abstract:** (50-100 words); Key Words: Biodiversity of terrestrial vertebrate from Vietnam

**Introduction:** Vietnam is known as a country of very rich biodiversity as well as world biodiversity hotspot, especially eco-region Truong Son Range and highland area. Recent studies show that Vietnam's nature still contains many un-discovered species of high conservation values. During 2007-2013, we have successfully conducted some collaborative project for study of biodiversity and development of conservation strategies for small mammals in center karst and highland of Vietnam.

**Result:** Recorded 182 mammal species of 32 families, 5 orders of which 12 species are identified as endangered and of high conservation priority. Discovery of 10 small mammal species including 5 bats such as *Kerivoula titania* Bate, Struebig, Hayes, Furey, Mya, Thong, Tien, Son, Harrison, Francis, and Csorba G., 2007; *Murina harpioloides* Kruskop and Eger, 2008; *Myotis phanluongi* Borisenko et al, 2008; *Murina beelzebub* Son, Furey and Csorba, 2011; *Myotis indochinensis* Son, Gorfol, Francis, Motokawa, Estok, Endo, Thong, Dang, Oshida, Csorba, 2013; 5 Shrews such as *Crocidura sokolovi* Jenkins, Abramov, Rozhnov and Makarova, 2007; *Crocidura zaisevi* Jenkins, Abramov, Rozhnov and Makarova, 2007; *Crocidura sokolovi* Jenkins, Abramov, Rozhnov and Makarova, 2007; Shrew *Crocidura zaisevi* Jenkins, Abramov, Rozhnov and Makarova, 2007; *Euroscaptor subanura* Kawada, Son, Can, 2012

**Discussion:** These findings again confirm high biodiversity of Vietnam's nature and contribute for conservation planning in Vietnam. The achieved results again confirm high biodiversity of Vietnam's nature and also indicated high effectiveness of collaboration.

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## PRE-CONGRESS LECTURE NOTE

### The Importance of Wildlife Medicine

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Key Words: Wildlife medicine, Conservation medicine, One-health, Bears, Education, JSZWM, ASZWM, Diploma

Wildlife medicine covers many fields of science and practical application, including disease ecology, control of infectious diseases and zoonoses, *ex situ* conservation using assisted reproductive techniques, rescue and rehabilitation, immobilization and handling, and so on. Recent many issues on wildlife medicine are associated with conservation issues and direct to aim of biodiversity conservation. Conservation medicine is an approach from wildlife medicine with an ultimate goal for conservation, and its concept is very similar to One-health, which integrates three kind of health; human health, animal health and environmental health leading to ecological health.

Unfortunately the current situation of biodiversity on the earth is not secured to be healthy, and its future seems to be pessimistic. Many species have been lost on the earth and huge numbers of species face to crisis of extinction due to human activity such as poaching/overhunting, habitat exploitation and environmental impact. One fourth of total mammalian species on the earth is categorized as threatened species by IUCN. We have to facilitate the approach from conservation medicine to conserve these threatened species.

In Hokkaido in which plenty wildlife including bears, deer, seals, whales, fox, raccoon dogs, marten, stoats, hares, bats, squirrels and many rodents is still maintained, wildlife issues such as human-wildlife conflicts, threat of endangered species, chemical pollution and invasive alien species are recognized recently. These problems should be resolved based on scientific data, and wildlife medicine is one of important scientific field for these problems. As a model of conservation practice of mammals, I will introduce a case of brown bears (*Ursus arctos*) in which some ecological and genetic research have been conducted.

In Japan, total 16 Universities have school of veterinary medicine, and undergraduate and graduate education on veterinary medicine has been conducted according to curriculum policy of each University. However, recently we established the model core-curriculum on undergraduate education of veterinary medicine, which includes 13 subjects. Each school has to have lectures which cover all of contents in this model core-curriculum and 2 years later, nationwide standard tests for all vet students will be assigned. We expect all of students have a minimum knowledge on wildlife medicine at the time of graduation.

Since the Japanese Society of Zoo and Wildlife Medicine (JSZWM) is founded in 1995, official journals fourth a year and newsletters twice a year have been published and many activity of committees and student session have been continuing with approximately 650 membership. The society founded the diploma system and about 10 people are working in each institute as experts certificated as diploma of wildlife medicine, zoo medicine, avian medicine and aquatic medicine.

Finally the Asian Society of Zoo and Wildlife Medicine (ASZWM) has been continuing annual meeting and already been raised diploma system. Furthermore we need to discuss more about future direction and integration which should lead to develop wildlife medicine and conservation medicine in Asian countries.

14 <sup>th</sup> OCT 2014 (Vietnam National University of Agriculture)			
9:00-12:00	ASZWM 2014 Pre-Educational Workshop		
14:00	Move to Tam Dao National Park Hotels after the Workshop in HUA (Bus from HUA )		
14 <sup>th</sup> OCT 2014 Evening (Green World Hotel at Tam Dao)			
17:00 – 18:00	Registration at Hotel (Green World Hotel, Tam Dao)		
18:30	Award Ceremony for ACCM Diploma Welcome Party hosted by ASZWM at Green World Hotel, Tam Dao		
15 <sup>th</sup> OCT 2014 (Green World Hotel at Tam Dao)			
9:00-10:00	Opening Session		
10:00-11:40	Special Session: What is Conservation Medicine? Room Pangolin		
11:40-12:30	Poster Presentation		
12:30-13:30	Lunch		
	Room Pangolin	Room Saola	
13:30-16:00	Welfare, Management and Conservation	13:30-15:15	Pathology
17:00-18:30	Zoo and Aquarium Vet Networking	15:30-16:45	Asian Elephant
18:30-	Welcome Party and Free Discussion for Asian Conservation Medicine; Announcement for 2015 Meeting / Dinner		
16 <sup>th</sup> OCT 2014 (Green World Hotel at Tam Dao)			
8:00-10:20	ASZWM-WDA Joint Session for Asian Wildlife Diseases Surveillance: Rabies in Wild Animals Room Pangolin		
10:35-12:05	Infectious Disease Room Pangolin		
12:05-13:45	Lunch		
	Animals Asia Bear Rescue Center	Room Pangolin	
13:45-18:00	Bear	14:00-16:30	Marine Mammals
19:00	Closing Ceremony (Award Ceremony, Announce of Next Meeting and Dinner)		
17 <sup>th</sup> OCT 2014			
Vietnam Biodiversity Workshop in Ha long City			
	Ha Long Bay ” Vietnam Biodiversity and Landscape Ecology” Workshop		