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Studies involving lead poisoning in swans have been conducted previously in many other countries, among them the U.S., Canada, the U.K. and Norway, but not many have been conducted in Japan. The primary way in which waterfowls become exposed to lead poisoning is through the ingestion of spent lead shots. The purpose of this study is to clarify the pathological features of lead poisoning in swans on the main island of Japan.

Twelve dead or euthanized swans in which lead poisoning was suspected, with a previous history of severe anorexia, inability to escape or stand, were examined. At necropsy, the vent area was stained with greenish watery diarrhea, and impacted crop with a large amount of grain was present. The presence of lead shots in the gizzard lumen was confirmed by x-ray in six swans, and all cases showed a dark greenish colored liver and distended gall bladder with dense bile contents. Microscopically, the most prominent finding was a marked deposition of brown hemosiderin pigment in the liver, spleen and kidneys in twelve swans, with the liver showing varying degrees of necrosis in eight swans. There were occasional intranuclear inclusion bodies in the liver cells of three swans. Chemical analysis showed high concentrations of lead in the liver and

kidneys. All of these symptoms and microscopic findings indicate that the most probable cause of death was lead poisoning, and chemical analysis of the lead content indeed revealed strong evidence of such poisoning.

Further international cooperative study to monitor lead poisoning in waterfowl is needed because most waterfowl including swans migrate plural countries. We are trying to make a network across countries.

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## [ Session II ] #7

### **Growth Profiles and Molecular Analyses of Recent Canine Distemper Isolates on Vero Cells Expressing Canine Signaling Lymphocyte Activation Molecule (SLAM)**

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Canine distemper virus (CDV) that is a *Morbillivirus* and belongs to the family *Paramyxoviridae* causes a highly infectious

and a systemic, often-fetal disease in dogs. So far, no cell lines have been adopted for routine titration of fresh tissues from CDV-infected animals. In this study, new cell line-Vero-DST cells had been used for isolation, titration of CDV.

Fresh samples of lymph node, lung and cerebrum taken post mortem from dogs No.1, 2 and 3 yielded canine distemper virus (CDV) strains 007Lm, 009L and 011C, respectively. They were titrated on Vero cells stably expressing canine signalling lymphocyte activation molecule (SLAM; Vero-DST cells). Growth curves of the three strains produced by titration of the released virus and cell-associated virus at various time points. All three isolates, especially 007Lm, grew well on Vero-DST cells. The titres of cell-associated virus of two strains (009L and 011C) were clearly lower than those of virus released into the culture supernate. The molecular and phylogenetic analyses of H and P gene reveal that the nucleotide and amino acid sequences of the genes of strain 007Lm after isolation in Vero-DST are identical to those of the origin virus from fresh tissue and strain 007Lm joins to the cluster of Asia 2 group of CDV strains that is distinct to the known clusters. The results indicate that 1) Vero-DST cells are not only useful for primary isolation but also efficient for titrating virus from fresh tissues and for the study of growth profiles of recent CDV isolates. 2) Strain 007Lm isolated from the vaccinated dog belongs to the cluster that is far from the vaccine strains in the phylogenetic trees of H and P genes.

## [ Session II ] #8

### **The Relationship Between AgNORs and PCNA Indices, Melan A Protein Expression, Histopathological Appearances and Clinical Factors as Prognostic Factor for Canine Oral Melanoma**

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The purpose of this research is to evaluate the relationship between histopathological appearances and clinical data, Argyrophilic Nucleolar Organizer Regions (AgNORS) and Proliferative Cell Nuclear Antigen (PCNA) indices and Melan A protein expression in canine oral melanoma. A retrospective study was performed on biopsy specimens of 67 dogs submitted from the year 2000 to 2003 of the Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University. Our results showed that average AgNORS index did not have significant differences with histopathological characteristics and Masson-Fontana Silver (MFS) stain but AgNORS index showed significant differences with histological grading and tumor diameter size ( $p < 0.05$ ).