GBST
Farm Animal Clinical Training and Research Center

The 4th Joint Symposium
between FACTRC in SNU and CADIC in UoM

New animal husbandry and veterinary technology

Center for Animal Disease Control,
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Date: 13:00-16:40, October 31, 2022

Venue: Room “Hidamari", Library 3rd floor, University of Miyazaki

Presentations (25 min presentation and 5 min discussion)

- 3 presenters from FACTRC

  Prof. Han Sang Yoo (Department of Infectious Diseases)

  Prof. Daesub Song (Department of Virology)

  Prof. Danil Kim (Department of Farm Animal Medicine)

- 3 presenters from CADIC

  Prof. Thi Thi Zin (Graduate School of Engineering)

  Assoc. Prof. Kentaro Yamada (Department of Veterinary Science)

  Assoc. Prof. Hirohisa Mekata (Center for Animal Disease Control)

Schedule

13:00-13:10 Brief address (Prof. Yoshida)

13:10-14:40 Session 1 (Chairpersons: Prof. Yoo, Prof. Yoshida)

1) Prof. Thi Thi Zin
   Cattle lameness detection using advanced digitization technologies.
2) Prof. Danil Kim
   Current status and future of Korean smart farm in cattle industry.
3) Assoc. Prof. Kentaro Yamada
   In vivo imaging of virus infection

14:40-15:00 Break

15:00-16:30 Session 2 (Chairpersons: Prof. Jang, Prof. Osawa)

4) Prof. Daesub Song
   Nanobiotechnology for diagnosis and vaccine against Disease X; unexpected emerging viral diseases.
5) Assoc. Prof. Hirohisa Mekata
   Single-Nucleotide Polymorphism on Spermatogenesis Associated 16 Gene-Coding Region Affecting Bovine Leukemia Virus Proviral Load.
6) Prof. Han Sang Yoo
   Current and Perspectives of *Mycobacterium avium* subsp. *paratuberculosis* infection.

16:30-16:40 Closing remarks (Prof. Jang)

Cattle Lameness Detection using Advanced Digitization Technologies

Thi Thi Zin, Tsubasa ONIZUKA, San Chain Tun, Su Larb Mon, Pyke Tin
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Abstract

Digitizing Livestock Farming is one of the current cutting-edge research projects for developing Precision Livestock Farming in the age of Agriculture 4.0 along with Digital Transformation (DX) technologies. In this concern, cattle lameness problem is the world third rank issue making seriously affect the health and welfare of dairy and beef cows. Moreover, the decrease in milk production and the increase in the calving interval of lame dairy cows increase costs for milk producers. Therefore, automated lameness detection could be useful, particularly in large herds, to provide a correct and early detection of lame cows in a timely manner and proper treatment. The idea behind automated lameness detection is to provide useful information that addresses an information gap, particularly with respect to mild and moderately lame cows. Among various lameness detection methods, we observed that a computer vision-based lameness detection method seems to be promising and being harmony with the advanced DX and AI (Artificial Intelligence) technologies.

Currently our research team is actively doing this cutting-edge cattle lameness research from variety of aspects to be harmony with 5G communication systems using 4K cameras. On the other hand, we have developed the various types of mathematical and statistical models for lameness detection by using visual and digital data through the collection from Depth Cameras. In doing so, we utilized all possible views such as top views, back views, front views, and side views.

Recently we have introduced a conceptual framework and operational simulation model for cattle lameness detection by using image depth data taken while cattle walking on the pathway from the milking center to the resting area. The proposed conceptual framework maps essential factors that detect lamed cattle based on body movement variability which is defined as the root mean square successive differences and geometric measures of the collected sequence of depth data during a field of depth camera view.

Furthermore, we also propose an intelligent method for detecting the lameness of dairy cattle by establishing a visual monitoring system using side view images taken by 4K camera on the laneways after milking process. We used Mask R-CNN method to segment the cattle region and then features are extracted from that region. Finally, the lameness scores classification is done by applying SVM (Support Vector Machine) classifier.

Our proposed image pattern variability measurement for lameness which we have preliminarily tested on real life dairy farm and received promising experimental results. In conclusion, we intended to explore and examine more challenging and practical research in lameness problems of modern livestock farming to be in line with advanced DX and AI technologies.

Keywords; Cattle Lameness Detection, Lameness Score, Cattle Region, Depth Data, Feature Extraction, Classification

This work is supported in part by “The Development and demonstration for the realization of problem-solving local 5G” from the Ministry of Internal Affairs and Communications and the Projects of “the On-farm Demonstration Trials of Smart Agriculture” from the Ministry of Agriculture, Forestry and Fisheries (funding agency: NARO).

Current status and future of Korean smart farm in cattle industry

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Digitalization is the process of converting analog information into digital form using an analog-to-digital converter. A broader concept, digital transformation, often expressed as DX, refers to the introduction of these digital technologies to institutions to improve efficiency or value. The first stage of DX was the time when digital infrastructure was built with the release of digital products in the late 1990s. The second stage was a digital business promotion stage that strengthened the digital business in the 2000s, a period when online shopping was actively developing. In the 3rd stage of DX, which is still in progress beyond the 2010s, advanced information and communication technology (ICT) platforms such as the internet of things (IoT), cloud, artificial intelligence (AI), and big data emerged, breaking away from the previous traditional industrial activities. Then, DX also began to accelerate. This period is also called the 4th industrial revolution era. The demand for smart farms is increasing due to population growth, climate change, the aging of the rural population, and automation.

Such ICT has also been applied to agriculture, and a smart farm refers to a place where this technology is applied. Smart farm technology was first introduced to the plant field, and the technology for sensing the environment and automatically controlling the environment inside the house was applied to provide an appropriate growth environment for crops. Also, in the livestock industry, especially in the industry of raising poultry and pigs that are raised in herds. smart farm technology has been introduced to sense the temperature, humidity, and air quality of the space where the animals are located and to control the environment suitable for the growth of animals. Automation technology has also been introduced in a way that reduces the labor previously provided by farmers. However, in the Korean cattle industry, where individual management is more important than herd management, only technology to control the environment of the pens is insufficient to improve the productivity. Therefore, products that individually evaluate the activity, body temperature, physiological state, etc. of cattle and provide information wirelessly have been developed and distributed, and as a result, the farmers themselves are reporting the improvement in productivity. The reason why these sensors can be actively introduced is that the government's smart farm distribution business played a big role. Currently, various sensors are being developed, and tests for application are in progress. In this presentation, the current status and future of smart farms in the Korean cattle industry will be discussed.

Keywords; Digital transformation, Smart farm, Sensor, Information and Communications Technology, Cattle industry

Session 1_3

In vivo imaging of virus infection

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Abstract

In vivo imaging is a noninvasive method that enables real-time monitoring of biological events in a small animal and now are widely used in biosciences including the field of virology. For *in vivo* imaging of virus infection, there are some advantages compared to the traditional virological approaches; i) viral replication and spread can be easily monitored semiquantitatively and longitudinally throughout the body of the same animal before any signs of disease appear, ii) the number of animals subjected to experiments can be reduced, iii) it is possible to identify unexpected but important sites of viral replication, and iv) a multimodal imaging enable to analyze virus-host interaction dynamics, such as virus replication and host immune responses.

The *in vivo* optical imaging using a fluorescent or bioluminescent reporter gene is often used. Each reporter has its advantages and disadvantages. In addition, it is known that wavelengths around 650–900 nm, the so-called 'near-infrared (NIR) window' or 'biological window,' are preferable for deep-tissue optical imaging. Recently, fluorescent and bioluminescent reporter genes that can emit the NIR light have been developed.

Rabies is a zoonotic disease and distributed globally except for several countries/regions including Japan. The causative agent is rabies virus (RABV), which belongs to the genus *Lyssavirus* in the family *Rhabdoviridae*. RABV causes fatal encephalitis after a long and variable incubation period, but there is still no diagnostics during the period. Moreover, although rabies is vaccine-preventable, there is no treatment after the onset (the case-fatality of nearly 100%).

Recently, we have established *in vivo* imaging system of RABV infection, and I would like to give some examples of the analysis. Furthermore, I will also present about development of an oncolytic mammalian orthoreovirus expressing an NIR fluorescent protein that can be applied to fluorescence-guided surgery.

Keywords; *in vivo* imaging, fluorescence, bioluminescence, near-infrared, rabies virus, mammalian orthoreovirus

Nanobiotechnology for diagnosis and vaccine against Disease X; unexpected emerging viral diseases

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Unlike in the past, emergences of viral infections have been and are occurring at very frequent intervals, causing enormous deaths and disability worldwide. Against a constant background of established infections, periodical emerging the epidemics of highly pathogenic influenza viruses (HPAI) greatly expand the global burden of infections. Accurate and rapid diagnosis of viral infections can result in effective and appropriate prevention and quarantine measures. This study compares and discusses about rapid diagnostics for the differential patho-typing between HPAI and LPAI using nanobiotechnology. The field of nanotechnology encompasses those technologies to fabricate materials, including sphere, cubic and nanoscale particles. Therefore, nanobiotechnology has the potential to offer not merely advances in diagnostics and vaccination to control infectious diseases but also in delivering various capabilities as below; Highly pathogenic avian influenza virus (HPAIV) infections have occurred continuously and crossed the species barrier to humans, leading to fatalities. A PCR-based molecular test is currently the most sensitive diagnostic tool for HPAIV; however, the results must be analyzed in centralized diagnosis systems by a trained individual. This requirement leads to delays in quarantine and isolation. To control the spread of HPAIV, rapid and accurate diagnostics suitable for field testing are needed, and the tests must facilitate a differential diagnosis between HPAIV and low pathogenic avian influenza virus (LPAIV), which undergo cleavage specifically by trypsin- or furin-like proteases, respectively. In this study, we have developed a differential avian influenza virus (AIV) rapid test kit and evaluated it in vitro and using clinical specimens from HPAIV H5N1-infected dogs. We demonstrated that this rapid test kit provides highly sensitive and specific detection of HPAIV and LPAIV and is thus a useful field diagnostic tool for H5N1 HPAIV outbreaks and for rapid quarantine control of the disease. In addition to diagnostics, development of better adjuvant accompanied with vaccine for enhancing immunogenicity has been greatly required for the control of influenza infection. Herein, we also address nano-complex of amphiphilic grafted poly (amino acid) and hydrophobic squalene (PA/S-NC) as a potent adjuvant that can act as a robust strategy for induce humoral (Th2) and cellular (Th1) immune responses as well as a delivery agent of antigens. CASq performed great biocompatibility, particle stability, and produced a high degree of antigen-specific antibodies and T cell immune responses in mice when CASq was co-administered with inactivated whole influenza virus antigen (CA04), in which CASq exhibited complete protection against lethal infection.
Keyword: HPAIV, nanobiotechnology, diagnostics, adjuvant, vaccination

Session 2_5

Single-Nucleotide Polymorphism on Spermatogenesis Associated 16 Gene-Coding Region Affecting Bovine Leukemia Virus Proviral Load

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Abstract

Bovine leukemia virus (BLV) is an etiological agent of malignant lymphoma in cattle and is endemic in many cattle-breeding countries. Thus, the development of cattle genetically resistant to BLV is desirable. The purpose of this study was to identify novel single-nucleotide polymorphisms (SNPs) related to resistance to BLV. A total of 146 DNA samples from cattle with high BLV proviral loads (PVLs) and 142 samples from cattle with low PVLs were used for a genome-wide association study (GWAS). For the verification of the GWAS results, an additional 1342 and 456 DNA samples from BLV-infected Japanese Black and Holstein cattle, respectively, were used for an SNP genotyping PCR to compare the genotypes for the identified SNPs and PVLs. An SNP located on the spermatogenesis associated 16 (SPATA16)-coding region on bovine chromosome 1 was found to exceed the moderate threshold ($p < 1.0 \times 10^{-5}$) in the Additive and Dominant models of the GWAS. The SNP genotyping PCR revealed that the median values of the PVL were 1278 copies/50 ng of genomic DNA for the major homozygous, 843 for the heterozygous, and 621 for the minor homozygous genotypes in the Japanese Black cattle ($p < 0.0001$). A similar tendency was also observed in the Holstein cattle. We found that cattle with the minor allele for this SNP showed 20-25% lower PVLs. Although the mechanisms through which this SNP impacts the PVL remain unknown, we found a novel SNP related to BLV resistance located on the SPATA16 gene-coding region on bovine chromosome 1.

Keywords; alleles; bovine leukemia virus; cattle; enzootic bovine leukosis; genome-wide association study; single-nucleotide polymorphism

Session 2_6

Current and Perspectives of *Mycobacterium avium* subsp. *paratuberculosis* infection

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Abstract

Mycobacterium avium subsp. *paratuberculosis* (MAP) is the causative agent of Johne's disease, a chronic debilitating disease in ruminants. MAP infection is characterized by chronic diarrhea, progressive wasting, and eventual death. MAP infection is also economically important because infected individuals exhibit weight loss and reduced milk production. The disease has been observed primarily in ruminants (e.g., cattle, sheep, goats, and deer) and various other nonruminant animals worldwide. In addition, the association of MAP with Crohn's disease, which is a type of chronic inflammatory bowel disease in humans, has been noted in numerous studies. To prevent and control the disease, it is very important to understand genetic characteristics of the causative agent and basic immunopathological mechanisms. Also, diagnosis of the early stage of the MAP infection has been received the attention.

To determine the genetic diversity of MAP, whole genome-based alignment and comparative analysis were performed using 40 publicly available MAP genomes, including newly sequenced Korean isolates. New genomic structures in MAP genomes and the genomic diversity of the MAP population were described by whole genome-based alignment and pangenome analysis, respectively. Two major types (C- and S-type) was identified by a phylogenetic tree based on the core genome and pangenome. However, B-type strains were discriminated from C-type strains. Also, functional analysis of the pangenome was performed using three virulence factor databases (i.e., PATRIC, VFDB, and Victors) to predict the phenotypic diversity of MAP in terms of pathogenicity. Based on the results of the pangenome analysis, a real-time PCR technique was developed to distinguish among S-, B- and C-type strains.

To understand immune evasion and the mechanism of persistence, the early phase interplays of the intracellular pathogens and their hosts was analyzed. Host-pathogen interactions at the transcriptomic level were investigated in an in vitro macrophage infection model. When differentiated human THP-1 cells were infected with MAP, the expression of various genes associated with stress responses and metabolism was altered in both host and MAP at 3 h post-infection. MAP upregulates stress-responsive global gene regulators, such as two-component systems and sigma factors, in response to oxidative and cell wall stress. Downstream genes involved in type VII secretion systems, cell wall synthesis (polyketide biosynthesis proteins), and iron uptake were changed in response to the intracellular environment of macrophages. On the host side, upregulation of inflammatory cytokine genes was observed along with pattern recognition receptor genes. Notably, alterations in gene sets involved in arginine metabolism

were observed in both the host and MAP, along with significant downregulation of NOS2 expression. These observations suggest that the utilization of metabolites such as arginine by intracellular MAP might affect host NO production. Our dual RNA-seq data can provide novel insights by capturing the global transcriptome with higher resolution, especially in MAP, thus enabling a more systematic understanding of host-pathogen interactions.

Infected animals excrete MAP via feces during the prolonged subclinical stage without exhibiting any clinical signs. Therefore, accurate detection of subclinical stage animals is crucial for successful eradication of JD in the herd. Several potential diagnostic methods have been proposed to detect early stage of MAP infection. We also carried out the analysis to find out new diagnostic method. I will discuss each of the methods in my presentation.
Those results from our researches suggest that the genetic diversity, immunopathological mechanisms and development new diagnostic methods might be useful to control and prevent the MAP infection. Based on the current status of researches of MAP infection, we should think about what it is the next steps in researches of MAP infection to control and prevent JD.

Keywords; *Mycobacteriums avium* subsp. *paratuberculosis*, genetic diversity, immunopathological mechanism, diagnosis, control and prevention