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は林水産省、宮崎県、日本獣医師会、8大学産業動物防疫コンソーシアム、

宮崎産業動物教育コンソーシアム委員会(拡幅事業推進検討会)



our experience from endemic areas Dr. Roman Pogranichniy (Kansas State University, USA)

> 2. Foot and mouth disease in Egypt: present status and future prospects *Dr. Abdelfattah Selim* (Benha University, Egypt)

3. Livestock pathogens in wildlife: establishment of the surveillance system and overview of its trials Dr. Sota Kobayashi (National Institute of Animal Health, NARO, Japan)

[Special session]

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2. Virulence and genetic characteristic of African swine fever virus isolated from Thailand, 2022–2023

Dr. Tapanut Songkasupa (National Institute of Animal Health, Thailand)

3. Environmental sampling to assess the bio-burden of *Mycobacterium avium* subspecies *paratuberculosis* in drylot pens on California dairies *Dr. Tapakorn Chamchoy*(National Institute of Animal Health, Thailand)

4. New technology for the elimination of foodborne pathogens from chicken carcasses using a high-pressure pulse jet spray system

Dr. Chanakan Chotiphutthikul (Veterinary Research and Development Center, Eastern Region, Thailand)

Closing Remarks: Dr. Ayako Yoshida (Director, Center for Animal Diseases Control, University of Miyazaki, Japan)

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Co-sponsor: Faculty of Agriculture, University of Miyazaki Special co-sponsor: Miyazaki Prefecture Tourism Association, MICE Supporter: Ministry of Agriculture, Forestry and Fisheries,

Miyazaki prefecture, Japan Veterinary Medical Association, The Consortium of 8 Universities of Animal Diseases Control, Miyazaki Industrial Animal Education Consortium Committee

宮崎大学産業動物防疫リサーチセンター センター長 吉田 彩子

現在、アジア諸国では口蹄疫やアフリカ豚熱の流行が深刻化し、国内への侵入が懸念されています。また、国内においても本州で豚熱の流行が拡大しており、九州における発生リスクも急激に高まってきています。このような悪性家畜伝染病の発生は、畜産業に甚大な経済的損失をもたらすだけでなく、食糧安全保障の観点からも国民生活に大きな影響を与えることから、産業動物防疫の重要性は今後さらに増大することが予測されます。

宮崎大学産業動物防疫リサーチャンター(CADIC)は、産業動物感染症を専門に扱う 国内唯一の研究機関であり、南九州という畜産が盛んな地域に立地し、地域の畜産関係者 や行政関係者、市民に産業動物感染症の最新の知見や対策法、課題をわかりやすく提供す ることを使命の一つとしています。特に、グローバル社会における海外悪性家畜伝染病の 情報共有は、伝染病コントロールのための重要課題であると考えています。

本シンポジウム第 1 部では、これらの悪性家畜伝染病に対する世界的な取り組みを紹 介し、CADIC が培ってきた防疫研究の成果を、これからの悪性家畜伝染病制御にいかに 役立てるのかについて、意見を交換する場を提供します。また、第2部では CADIC と共 同研究を行なっているタイの動物感染症研究機関から、産業動物防疫に関する研究内容を 紹介します。

国立大学法人 宮崎大学 学長

鮫島 浩

本日、宮崎大学産業動物防疫リサーチセンター主催の第13回国際シンポジウム「食料 安全保障と持続可能な畜産のために私たちは何をすべきか?」を開催するにあたり、開会 のご挨拶を申し上げます。

近年では、異常気象やウクライナ危機を起因とする飼料の高騰を受け、畜産の生産コス トが上昇しています。また、近隣諸国では口蹄疫等の重要家畜伝染病の発生が続いてお り、我が国への侵入リスクが高まっています。国内においては、牛伝染性リンパ腫といっ た消耗性疾患を引き起こす持続感染症がまん延しています。家畜伝染病の発生は生産性の 低下といった直接的な経済損失のみならず、畜産物貿易にも深刻な影響を与えます。この ような課題に対し、宮崎大学産業動物防疫リサーチセンターは、病原体に対する基礎研究 から、疫学、診断、予防といった応用研究まで幅広く取り扱い、家畜伝染病を多面的なア プローチで解決する実践型の研究に取り組んでいます。また、人材の養成や近隣諸国との 情報交換、海外からの研修の受け入れなどを行っています。

今回のシンポジウムでは、口蹄疫はもちろん、病原体を媒介する野生動物に関する調査 のあり方、国内ではまだ発生したことのないアフリカ豚熱について、活発な意見交換を行 いたいと思っています。また、特別セッションとして、本学が取り組んでいる地球規模課 題対応国際科学技術協力プログラム (SATREPS) についてもご紹介させていただきます。

結びに、本日のシンポジウムが、ご参加くださった皆様にとって、有意義なものとなり、 家畜防疫について議論を深める機会となることを期待いたしますとともに、皆様の益々の ご活躍を祈念して、開会の挨拶といたします。

Program

9:50-10:00 Opening Remarks Dr. Hiroshi Sameshima, President, University of Miyazaki

Master of the symposium; Dr. Takako Taniguchi, CADIC, UOM

Part I

(10:00-12:00)

Chairperson: Dr. Tamaki Okabayashi, Dr. Hirohisa Mekata, CADIC, UOM 10:00-11:00 Prevention of African Swine Fever from entry into commercial farms: our experience from

endemic areas Dr. Roman Pogranichniy, Kansas State University, USA

11:00-12:00

Foot and mouth disease in Egypt: present status and future prospects Dr. Abdelfattah Selim, Benha University, Egypt

12:00-13:30 Lunch time (90 min)

Chairperson: Dr. Kentaro Yamada, Dr. Akatsuki Saito, Faculty of Agriculture, UOM 13:30-14:30

Livestock pathogens in wildlife: establishment of surveillance system and overview of its trials Dr. Sota Kobayashi, National Institute of Animal Health, NARO, Japan

Part II

(14:30-17:15)

14:30

Greeting

Science and Technology Research Partnership for Sustainable Development (SATREPS) Dr. Lerdchai Chintapitaksakul, Director of National Institute of Animal Health, DLD, Thailand

14:35-14:45

Overview of the SATREPS project that CADIC has been tackling Dr. Naoaki Misawa, University of Miyazaki, Japan

14:45-15:25

Virulence and genetic characteristic of African swine fever virus isolated from Thailand, 2022–2023

Dr. Tapanut Songkasupa, National Institute of Animal Health, Thailand

15:25-15:45 Coffee break (20 min)

Chairperson: Dr. Atsushi Iguchi, Dr. Ryoko Uemura, Faculty of Agriculture, UOM 15:45-16:25 Environmental sampling to assess the bio-burden of *Mycobacterium avium* subspecies *paratuberculosis* in drylot pens on California dairies

Dr. Tapakorn Chamchoy, National Institute of Animal Health, Thailand

16:25-17:05

New technology for the elimination of foodborne pathogens from chicken carcasses using a highpressure pulse jet spray system Dr. Chanakan Chotiphutthikul, Veterinary Research and Development Center, Eastern Region, DLD, Thailand

17:05-17:15 Closing Remarks Dr. Ayako Yoshida, Director & Professor, Center for Animal Diseases Control, UOM

Prevention of African Swine Fever from entry into commercial farms: our experience from endemic areas

Roman Pogranichniy DVM, PhD

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While African swine fever (ASF) prevention is so important for swine producers around the world, customized recommendations based on the situation on each farm are needed. The goal of our studies was to identify biosecurity gaps to reduce outbreaks of ASF in Vietnam and Ukraine. The projects were focused on helping swine producers to prevent ASF entry into farms and identifying risk factors of virus introduction on the farms. This work was done in collaboration with swine farm owners and veterinarians on farms in Vietnam and Ukraine that operate in an endemic area for ASF. We designed surveys for biosecurity on the swine farms to work with interested swine farm owners and veterinarians.

Data was collected via a survey based on questions from the outbreak investigation instrument. Several major categories were examined on an individual production level including swine movement, pickup and deliveries, people movement, pork/food product entry, manure removal, domestic/wild animals and insects, and air/water. Each major category was then broken down into several subcategories, and these subcategories were rated on a scale of 0.00 to 1.00 with 0.00 being areas in which there is least risk and 1.00 being the most vulnerable (high risk) to the introduction of ASFV to the farm. The top ten shared risks to the spread of ASF included the entry of water, replacement animals import and testing, equipment used by on-farm employees, semen testing and handling, and sanitary transportation. These reports were generated only upon full completion of surveys and allow producers to understand the specific practices that are the greatest risk for ASFV introduction into their farms so tailored biosecurity measures can be put in place. We analyzed results from a large number of farms located in endemic areas. A report and analysis was generated and sent back to the person who submitted answers to the questions that we asked in the survey. The subcategory results from completed surveys were then ranked as higher risk (>0.50) and lower risk (≤ 0.49) and compiled into reports that highlighted areas that would most benefit from specific biosecurity actions to reduce the risk of a farm introducing ASF to its herds.

The top ten shared risks among the 36 completed farm surveys identified to date included feed trucks, replacement breeding animals, semen, entry of water, visitors, tools (both for breeding replacements and cull animals), livestock trailers (for both weaned pigs and replacement pigs) as well as pork and other food entering the premises. It was up to the swine owners and the management team to work on improving the biosecurity on the farm and make improvements to prevent virus entry. Prevention of the spreading of ASF between farms, regions, or countries is a team effort. This work conducted by many collaborators involved in the projects in Ukraine and Vietnam will be presented.

Keywords: ASF, Biosecurity, Virus prevention

Foot and mouth disease in Egypt: present status and future prospects

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The foot and mouth disease (FMD) virus affects livestock all around the world. Seven distinct serotypes exist, and each has a variety of topotypes, genetic lineages, and strains. FMD is one of the most contagious diseases in cloven hoof animals, and it remains a significant impediment to agricultural growth in endemic nations. Despite the low mortality rate from FMD have been reported in adult animals, immediate losses from poor milk and meat output, treatment costs, and restrictions on the trade in animals and animal products during epidemics have a substantial economic impact. The FMD considered as one of endemic disease in Egypt with three strains A, O, and SAT2 in the last 65 years. The first FMD epidemic was recorded in Egypt in 1950, and it was caused by serotype SAT2. Egypt had several FMD outbreaks during 1958 with serotypes A and O, while serotype O have been reported in 1961, 1964, 1965, 1970, 1974, 1983, 1987, 1989, 1993, and 2000. Serotype A reinvaded again in 1967 and 1972. Also, serotype A caused multiple outbreaks in Egyptian governorates including Ismailia, Alexandria, Behera, Cairo, Dakahlia, Dumyat, Fayum, and Menofia in February 2006. Furthermore, SAT2-Iranian strain was discovered in 2012, followed by SAT2-Libyan strain detection in 2018. Despite the fact that vaccination is mandatory in Egypt, outbreaks of FMD continue to occur throughout the country. The discovery of novel FMD variations in the country has prompted concerns about the genetic variety of these variants, as well as the need for a better understanding of these variants to establish an efficient control program and choose the most reliable vaccine.

Keywords: FMD, Egypt, Epidemiology, Serotype A, Serotype O, Serotype SAT2

Livestock pathogens in wildlife: establishment of surveillance system and overview of its trials

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In considering the prevention of various pathogens from entering livestock and poultry farms, the necessity to focus on environmental routes via wildlife, as well as human-caused paths including humans, goods, vehicles, etc. has long been recognized. Therefore, to help promote on-farm infectious disease control, it is becoming increasingly important to know the prevalence of livestock pathogens in wildlife. Moreover, as on-farm Biosecurity Standards mention measures against wildlife, farmers are required to take necessary actions daily. For these reasons, it is desirable to establish and maintain a wildlife surveillance system that can ascertain the situation.

Finding the very first cases of rare diseases in the country and the first case of specific diseases in the season is essential for providing new and early alerts to the livestock industry. However, in the case of endemic and/or chronic diseases, it is also necessary to maintain long-term surveillance to understand the extent to which they are infesting the environment (i.e., how much livestock professionals should be concerned about them) rather than determining their presence. In this respect, some one-off trials have been carried out thus far. However, as most of these trials were conducted in specific small areas, if the results are used in the risk assessment as national data, it will be difficult to obtain valid conclusions due to biases.

Given these conditions, the National Institute of Animal Health has been conducting nationwide surveillance of wild boars and deer for livestock pathogens since 2014 under a project supported by the Ministry of Agriculture, Forestry and Fisheries. This presentation will introduce the establishment and maintenance of the current surveillance system, as well as provide an overview of the results obtained thus far.

Keywords: Surveillance, Livestock pathogen, Wildlife

Overview of the SATREPS project that CADIC has been tackling

Naoaki Misawa

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SATREPS is a science and technology diplomacy initiative that promotes international joint research using advanced science and technology from Japan in combination with Official Development Assistance (ODA). The program is a collaboration between JST, the Japan International Cooperation Agency (JICA) and the Japan Agency for Medical Research and Development (AMED) supported by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) and the Ministry of Foreign Affairs (MOFA). Based on the needs of developing countries, JST and JICA cooperate to promote international joint research targeting global issues with the objective of utilization of research outcomes. Implemented through collaboration with ODA, the aim of the program is to acquire new knowledge and technology that lead to the resolution of global issues and the advancement of science and technology, and through this process, to create innovations. Also, SATREPS will actively correspond to the Sustainable Development Goals (SDGs) and contribute to the international community. Based on this concept of the SATREPS, in FY2020, we started a collaborative research project with Thailand, "The Project for the Acceleration of Livestock Revolution in Thailand aiming to be the Kitchen of the World through the Development of Novel Technologies for Stable Livestock Production and Food Safety". Foot-and-mouth disease (FMD) and other major livestock infectious diseases are causing export restrictions and a decline in livestock production. This project, in Thailand who aims to be the Kitchen of the World, applies experiences learned from controlling FMD outbreak in Miyazaki Prefecture. In cooperation with government agencies and veterinary universities, a multi-diagnostic system for major infectious livestock diseases and a food poisoning bacteria elimination technology from the poultry are to be developed, and an epidemic prevention system based on the disease dissemination mathematical model is to be established. Through these research and training programs, this project strives to foster experts who can contribute to livestock epidemic prevention in the globalized era. This project establishes technology for controlling livestock infectious diseases such as FMD in Thailand. Thailand, who plays a leading role in ASEAN, will influence the neighboring countries after implementing this technology which consequently contributes to a stable and safe livestock supply worldwide.

Keywords: FMD, Food safety, New diagnoses, SDGs, Transboundary infectious diseases

Virulence and genetic characteristic of African swine fever virus isolated from Thailand, 2022 – 2023

 Tapanut Songkasupa¹, Nuttun Pengpetch¹, Kultyarat Bhakha¹, Kanokwan Puangjinda¹, Marutpong Pumpuang², Prakit Boonpornprasert¹, Bundit Nuansrichay¹

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African swine fever (ASF) is a deadly viral disease that infects domestic pigs and wild boars. Since 2018, the disease has emerged in China and rapidly spread throughout Asian countries causing tremendous socioeconomic consequences. This study aims to determine the severity, genotype, and genetic characteristics of representative ASF viruses (ASFV) responsible for the outbreaks in 2022-2023 in Thailand. The genome of the ASFV isolated during the first outbreak in Thailand was analyzed. The ASF/Pig/Nakhonprathom/Thailand/NIAH-NPT1/2022 was 189,404 base pairs. To evaluate severity of ASFV, specific-pathogen-free pigs intramuscularly inoculated with different virus dosages at 10¹-10^{6.5} HAD50 exhibited high pyrexia, skin discoloration and death. The incubation periods were 2-8 days post inoculation (dpi) and inoculated pigs at $10^{1}-10^{6.5}$ HAD50 died between 5-19 dpi. Viremia was found at 3dpi. and ASFV genome was detected from nasal and rectal swab samples on 3-5 dpi. For molecular characterization, the amplification and sequencing of the DNA fragments of the putative B646L gene encoding the major capsid protein p72, and intergenic region (IGR) between the 173R and I329L genes were performed. Phylogenetic analysis revealed that all ASFV isolates circulating in Thailand belongs to genotype II, central variable region (CVR) type I and intergenic region (IGR) II. These results indicate that Thai ASFVs are highly virulent and transmissible in domestic pigs. Our study demonstrates the threat of ASFV and emphasizes the need to control and eradicate ASF in Thailand.

Keywords: ASF, African swine fever, Thailand, Virulence

Environmental sampling to assess the bio-burden of *Mycobacterium avium* subspecies *paratuberculosis* in drylot pens on California dairies.

Tapakorn Chamchoy

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Mycobacterium avium subspecies paratuberculosis (MAP) is a bacterium that can cause substantial economic losses in infected dairy herds due to reduced milk production and increased cow-replacement costs. In order to control MAP in dairies with drylot pens, a standardized environmental sampling protocol to quantify MAP in fecal slurry was developed based on an existing protocol for freestall pens. Specifically, following a 24-hour hold of the flush, a grab sample of approximately 10ml of fecal slurry was collected every 1 meter along the flush lane of the drylot pens, avoiding individual cow fecal pats. To determine the reliability and repatability of the new environmental sampling protocol for estimation of MAP bioburden at the pen level, two collectors simultaneously collected fecal slurry samples every day for 3 days from six drylot cow pens on two Central California dairies. During the study period no cow movement between pens was allowed with the exception of sick cows. The study herds had MAP seroprevalence of 5.8% and 3.2%, respectively, based on whole pen serum ELISA results. Variance components models for quantitative real-time PCR (qPCR) results showed samples collected from different pens on different dairies accounted for greater variablity in MAP concentration (65%), while samples collected by different collectors had the least variability (0.1%). In contrast, variability in MAP concentration in environmental samples collected on different days had 25% variability. The intraclass correlation coefficient (ICC) showed high reliability (93%) of environmental sampling simultaneously by different collectors. In contrast, the reliability of environmental sampling at different days was 65%, which was similar to the reliability for sampling by different collectors on different days. Investigators can expect high reliability when employing the new environmental sampling protocol along with qPCR testing of environmental samples from drylot pens.

Keywords: Environmental Sampling, Mycobacterium avium subspecies paratuberculosis, Quantitative real-time PCR, Drylot pen, Intraclass correlation coefficient

New technology for the elimination of foodborne pathogens from chicken carcasses using a high-pressure pulse jet spray system

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Salmonellosis is a zoonotic infection caused by Salmonella enterica subsp. enterica is an important substantial public health threat worldwide as one of the most common causes of foodborne infection. It is often associated with the consumption of contaminated poultry products such as chicken. Since chicken carcasses can become contaminated with Salmonella during the processing stage at a processing plant due to poor hygiene practices, inadequate sanitation, or crosscontamination. Some disinfectants widely are used in many countries to reduce Salmonella contamination of chicken carcasses in processing plants. However, the bactericidal efficacy of Salmonella is not significant. In this study, we evaluated an alternative strategy to reduce contaminated bacteria by using a high-pressure pulsed jet spray (HPPJS) which washes out the bacteria attached to a carcass. In a preliminary study, chicken carcasses were artificially inoculated with Salmonella Enteritidis (ATCC 13076) and followed by spraying using the HPPJS under various conditions. The treatment groups were divided into two groups, based on exposure time (5 or 15 sec), and treated with tap water or peracetic acid (PAA) with 0.05% or 0.07%. Salmonella counts were determined using the most-probable-number (MPN) method. Total Coliform (TC) and Aerobic Plate Count (APC) from the carcass skins were examined by a direct plate count method. The efficacy of HPPJS was evaluated by comparison of the number of bacteria before and after treatment by HPPJS. The present study showed a significant reduction in Salmonella only when the chicken carcasses were sprayed with 0.05% PAA for 5 sec. While a significant reduction of APC was observed in most conditions, there was no significant reduction of TC in any conditions. However, further study has already been planned and conducted to improve the efficacy of HPPJS and optimize the conditions for decontamination. Since this technology would effectively remove Salmonella from contaminated chicken skin, this research contributes to improving food safety practices in the poultry industry.

Keywords: Bacterial contamination, Chicken carcass, High-pressure pulsed jet spray (HPPJS), Pathogen reduction, Salmonella, Spraying systems