The 3rd Thailand - Japan Joint Conference on Animal Health 2014

July 16-18, 2014
National Institute of Animal Health, Bangkok, Thailand

PROCEEDINGS

“Thailand-Japan Animal Health Research”
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Preface

On behalf of the National Institute of Animal Health of Thailand (NIAH-Thailand) and the Conference Organizing Committee, it is our great pleasure to welcome you all to “the 3rd Thailand – Japan Joint Conference on Animal Health 2014” (the 3rd TJJC 2014) held from 16 to 18 July 2014 at NIAH-Thailand in Bangkok. This Joint Conference has been organized annually under the MOU signed in May 2012 between the Department of Livestock Development (DLD) of Thailand and the National Institute of Animal Health of the National Agriculture and Food Research Organization (NIAH-NARO) of Japan. The 1st TJJC 2012 held in Bangkok and the 2nd TJJC 2013 held in Tsukuba were successful in achieving their objectives. Our young generation researchers had an opportunity to present their researches, exchange ideas and make new contacts. Moreover, a collaborative project has been discussed and initiated.

The theme for this year’s conference, “Thailand – Japan Animal Health Research”, was chosen as a means of bringing together the researchers, scientists and veterinarians from NIAH-Thailand and NIAH-Japan to share knowledge, experiences, and technology advances in term of animal disease research and diagnosis, to strengthen relationship and academic collaboration between the two institutions.

More than 100 participants from Japan and Thailand attend this year’s conference, which consists of 20 oral and 21 poster presentations in addition to 3 invited talks by distinguished keynote speakers including Dr. Tomoyuki Tsuda, the Director General of NIAH-Japan, Prof. Dr. Naoaki Misawa, the Director of Center for Animal Disease Control, University of Miyazaki and Dr. Thanawat Tiensin, Head of International Livestock Trade and Regulations Group, Division of International Livestock Cooperation, DLD-Thailand.

I would like to express my sincere gratitude and acknowledgements to all the following people who contribute to the 3rd TJJC 2014: the distinguished keynote speakers, the authors of all submitted papers, the chairpersons of each session, our honorable guests and participants for their time, effort and assistance without their cooperation this conference would not be possible. Last but not least, my special thanks go to the Japanese contact persons and the conference organizing committee for their hard work and effort in making this conference a valuable and memorable experience.

Finally, I sincerely hope that our scientific and social programs will stimulate the advancement of knowledge and our good friendship, and also hope that the enthusiasm and fellowship generated among participants at the conference this year will be long remembered.

Best wishes,

Dr. Preecha Wongwicharn
Director of the National Institute of Animal Health, Thailand
July 2014
Wednesday July 16, 2014

08.00 a.m. - 08.30 a.m. Registration
08.30 a.m. - 08.50 a.m. Welcome address
   By Dr. Preecha Wongwicharn
   Director
   NIAH, THAILAND

Opening address
By Dr. Tritsadee Chaosuancharoen
Director General
DLD, THAILAND

08.50 a.m. - 09.20 a.m. Keynote speaker I: "Thailand – Japan animal health research"
   By Dr. Tomoyuki Tsuda
   Director General
   NIAH, JAPAN

09.20 a.m. - 09.30 a.m. VDO presentation
09.30 a.m. - 09.45 a.m. Present a token of appreciation
09.45 a.m. - 10.00 a.m. Group photo
10.00 a.m. - 10.20 a.m. *** Coffee/Tea break ***
10.20 a.m. - 10.50 a.m. Keynote speaker II: "The global harmonization for animal epidemic prevention strategies -Approaches for one world, one health-"
   By Dr. Naoaki Misawa
   Director of Center for Animal Disease Control (CADIC)
   University of Miyazaki, JAPAN

Oral presentation, Session A
Chairperson: Dr. Ladda Trongwongsa

10.50 a.m. - 11.10 a.m. Experimental infection of animals with a foot-and-mouth disease virus isolated from the 2010 epidemic in Japan.
   Katsuhiko Fukai, Kazaki Morioka, Manabu Yamada, Kazuo Yoshida, Tatsuya Nishi, Rie Kitan, Reiko Yamazoe, Toru Kanno

11.10 a.m. - 11.30 a.m. Molecular epidemiology surveillance of foot and mouth disease virus type A in Thailand during 2012-2014.
   Panithan Thongtha, Arongkorn Panhumart, Rattanee Thongth, Somjai Kamolsiripichai pomp

11.30 a.m. - 11.50 a.m. Transition of bovine viral diarrhea virus subgenotypes in Japan.
   Ken-Ichiro Kameyama, Misako Konishi, Takamitsu Tsuob, Makoto Yamakawa

11.50 a.m. - 12.10 p.m. Detection of bovine leukemia virus proviral DNA associated with lymphoma by in situ hybridization technique.
   Nawaporn Tearning, Thitikan Jirakittisonth, Thairka Chantamanechote, Vara Varong, Jesada Ratthanophart, Ruennrudee Bunyhotara, Tuangthong Patchimasiri

12.10 p.m. - 01.00 p.m. *** Lunch ***

Oral presentation, Session B
Chairperson: Dr. Chanpen Chamnanpood

01.00 p.m. - 01.20 p.m. Avian paramyxoviruses are vaccine vector candidates that escape immunity conferred by NDV vaccination in chickens.
   Ryota Tsunekuni, Hirokazu Hikono, Takehiko Saito

01.20 p.m. - 01.40 p.m. Genetic variation of infectious bronchitis virus in southern Thailand.
   Narupol Promkuntod, Sasadum Pongme, Sayan Yoidam

01.40 p.m. - 02.00 p.m. Genetic analysis of an H5N8 highly pathogenic avian influenza virus isolated from chickens in Japan in 2014.
   Katushi Kanehira, Yuko Uchida, Nobuhiro Takamay, Hirokazu Hikono, Ryota Tsunekuni, Hirokazu Hikono, Takehiko Saito

02.00 p.m. - 02.20 p.m. Application of the PigINFO benchmarking system to analyze effects of PRRS virus on herd productivity.
   Itsuro Yamane, Sayoo Ishizeki, Hisanori Yamazaki

02.20 p.m. - 02.40 p.m. Pathogenicity of porcine reproductive and respiratory syndrome virus isolated in Japan.
   Michihiro Takagi, Suryo Purnomo Edi, Nachiko Hattori, Yojiro Taniuchi, Kazufumi Kuga, Hiroshi Ita, Tomooyuki Shibahara, Osamu Mikami, Mitsutaka Ikezawa, Kenji Kawashima

02.40 p.m. - 03.00 p.m. A case report: Orf virus in goats in Chonburi province.
   Nimit Choe-ngern, Akachai Tunak, Jiraporn Lueanjan, Mutita Chalamaat, Marisa Bintavithok

03.00 p.m. - 03.20 p.m. *** Coffee/Tea break ***
Oral presentation, Session C
Chairperson: Dr. Pornpen Pathanasophon

03.20 p.m. - 03.40 p.m. Multilocus sequence typing analysis of *Melissococcus plutonius* isolated from European and Japanese honeybees: Spread of some sequence types across borders and bee species.  
*Daisuke Takamatsu, Keiko Morinshi, Aya Sakamoto, Masatoshi Okura, Makoto Osaki*

03.40 p.m. - 04.00 p.m. Characterization of virulence-resistance plasmids carried by emerging multidrug resistant *Salmonella* Typhimurium isolated from cattle in Hokkaido, Japan.  
*Yukino Tamamura, Kiyoshi Tanaka, Ikuo Uchida*

04.00 p.m. - 04.20 p.m. Mapping of Q fever in ruminants, Thailand during 2012 - 2013.  
*Pattarin Opaschaitat, Tippa Wongstitwilairoong, Surapong Wongkasemjit, Vimon Jirathanawat, Preecha Wongwicharn, Samuel L. Yingst*

04.20 p.m. - 04.40 p.m. Role of cell-surface molecules of *Erysipelothrix rhusiopathiae* in adherence to porcine endothelial cell.  
*Yoshihiro Shimoji, Yohsuke Ogawa, Masahiro Eguchi, Masumi Sata*

04.40 p.m. - 05.00 p.m. Development of a novel PCR-based method for typing of capsular polysaccharide synthesis gene clusters of *Streptococcus suis*.  
*Masatoshi Okura, Claude Lachace, Makoto Osaki, Tsutomu Sekizaki, Fumito Maruyama, Takashi Nozawa, Ichiro Nakagawa, Shigeyuki Hamada, Celine Rossignol, Marcelo Gottschalk, Daisuke Takamatsu*

06.00 p.m. - 09.30 p.m. Welcome party

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**Thursday July 17, 2014**

09.10 a.m. - 09.40 a.m. Keynote speaker III: “Transboundary animal diseases and food safety issues: Challenges and opportunities for capacity building of veterinary services and research”  
By Dr. Thanawat Tiensin  
Head of International Livestock Trade and Regulations Group  
Division of International Livestock Cooperation, DLD, THAILAND

09.40 a.m. - 10.00 a.m. *** Coffee/Tea break ***

Oral Presentation, Session D
Chairperson: Dr. Patchima Indrakamhang

10.00 a.m. - 10.20 a.m. Seroprevalence and risk factors of *Toxoplasma gondii* in goats and sheep in the western Thailand.  
*Philaiphon Chetiyawan, Jasada Julagaivansujarit, Trakansak Paethaisong*

10.20 a.m. - 10.40 a.m. External parasites of cultured freshwater fish in central region of Thailand during 2011 - 2013.  
*Juntra Wattanamethanont and Sineepan Puvanan*

10.40 a.m. - 11.00 a.m. Development of portable near-infrared (NIR) instruments and system for rapid and easy measurement of blood packed cell volume (PCV) in cattle.  
*Yutaka Terada, Akifumi Ikehata, Xuan Luo, Kunio Sashida, Shanji Park*

11.00 a.m. - 11.20 a.m. Nitrate poisoning due to ingestion of *Mimosa invisa* in cattle.  
*Jamras Lerdrei, Aroonpan Doongsongnern, Udom Chuachan*

11.20 a.m. - 11.40 a.m. Changes in the concentration of fumonisins in forage rice during the growing period, differences among cultivars and identification of the causal fungus.  
*Ryuichi Jegaki, Masanori Tohno, Kohji Yamamura, Takao Tsukiboshi*

11.40 a.m. - 12.00 p.m. Closing the conference

12.00 p.m. - 01.00 p.m. *** Lunch ***

01.00 p.m. - 02.00 p.m. Poster Session

02.00 p.m. - 03.00 p.m. Japan – Thailand NIAH Meeting

03.00 p.m. - 03.20 p.m. *** Coffee/Tea break ***

03.20 p.m. - 04.30 p.m. Laboratory visit

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**Friday July 18, 2014**

06.45 a.m. - 12.00 a.m. Field trip

12.00 a.m. - 01.00 p.m. Lunch

01.00 p.m. - 06.00 p.m. Field trip (cont')
EDITOR : Dr. Sontana Mimapan

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WELCOME ADDRESS

By Dr. Preecha Wongwicharn
Director of National Institute of Animal Health
at Opening Ceremony
The 3rd Thailand - Japan Joint Conference on Animal Health 2014
at 8.30 AM on 16 July 2014
Conference Hall, National Institute of Animal Health, Bangkok

Dr. Tritsadee Chaosuancharooen, Director General of Department of Livestock Development
Dr. Tomoyuki Tsuda, Director General of the National Institute of Animal Health, Japan
Dr. Naoaki Misawa, Director of Center for Animal Disease Control, University of Miyazaki

Dear researchers from NIAHs of Japan and Thailand
Honorable guests, participants, ladies and gentlemen

On behalf of NIAH-Thailand, may I welcome Dr. Tomoyuki Tsuda, Director General of NIAH-Japan and Dr. Naoaki Misawa, Director of Center for Animal Disease Control, University of Miyazaki, all honorable guests and participants to Thailand and to the Opening Ceremony of the 3rd Thailand - Japan Joint Conference on Animal Health 2014. This conference is the result of successful cooperation between NIAHs of Japan and Thailand under the MOU signed in 2012. Before that our long relationship started from the technical cooperation project kindly supported by the Japanese Government through JICA project. As a result, NIAH-Thailand has revealed its capabilities in diagnosis of animal diseases, performing research and technology transfer for Thailand and neighboring countries.

The objectives of the conference are to strengthen relationship and academic collaboration between NIAH-Japan and NIAH-Thailand and to share knowledge, experiences, and technology advances in term of animal disease research and diagnosis between the two institutions.

The conference will cover 3 invited talks by distinguished keynote speakers including Dr. Tomoyuki Tsuda, Dr. Naoaki Misawa and Dr. Thanawat Tiensin, 20 oral presentations and 21 poster presentations. In addition, on the second day, there will be a meeting session for Japanese and Thai researchers to discuss together for the future collaboration. And last but not least, we organize a special one-day trip for our Japanese friends to look around Thai scenery and culture on the third day.

There are approximately 120 participants attending this conference consisting of researchers, scientists, veterinarians, and retired officers and experts from Japan and Thailand.

We hope that this conference will provide a crucial benefit to all parties involved and make a new pathway to strengthen the development of our capacity building.

Ladies and gentlemen, may I now request Dr. Tritsadee Chaosuancharooen, Director General of the Department of Livestock Development to give the opening address.

Thank you.
OPENING ADDRESS

By
Dr. Tritsadee Chaosuancharoen
Director General of the Department of Livestock Development
at Opening Ceremony
The 3rd Thailand - Japan Joint Conference on Animal Health 2014
at 8.30 AM on 16 July 2014
Conference Hall, National Institute of Animal Health, Bangkok

Dr. Tomoyuki Tsuda, Director General of the National Institute of Animal Health, Japan
Dr. Naoaki Misawa, Director of Center for Animal Disease Control, University of Miyazaki
Dr. Preecha Wongwicharn, Director of National Institute of Animal Health
Dear researchers from NIAHs of Japan and Thailand
Honorable guests, participants, ladies and gentlemen

On behalf of the Department of Livestock Development, I wish to express my gratitude to Dr. Tomoyuki Tsuda, Director General of NIAH-Japan and Dr. Naoaki Misawa, Director of Center for Animal Disease Control, University of Miyazaki for not only being our honorable guests at the opening ceremony of the 3rd Thailand - Japan Joint Conference on Animal Health 2014, but also being our keynote speakers.

At the present time, it’s apparently that NIAH-Thailand is highly competence in animal health-related issues. We have a great potential to proudly cooperate with other government units associated with public health, education and public services at both national and international levels. Moreover, when the newly emerging and re-emerging diseases occur as a cause of the international problems, we are capable to inquire into the cause of those diseases and effectively coordinate with related sectors to control and eradicate the infections. Therefore, Thailand has been able to cope with and saved from several regional and global outbreaks of serious epidemic diseases. It is undeniable that our success in laboratory and field works are based on the basic and advanced academic knowledge as well as technologies contributed from NIAH-Japan. I am pleased that the two institutions have opportunity to work together again through this annual academic knowledge exchange and sharing conference. I have learned that the second Thailand-Japan Joint Conference in Tsukuba last year was successful in achieving its objectives. I believe that the conference this year will not only provide benefits to our young generation researchers, but it will also maintain the existence of the excellent relationship forever.

I would like to take this opportunity to express my sincere appreciation to NIAH-Japan and University of Miyazaki for your kind cooperation. Moreover, I would like to thank the organizing committee for their time and effort in hosting this conference. I wish the conference will be successful and make a mutual benefit to all people involved. May I now declare the 3rd Thailand - Japan Joint Conference on Animal Health 2014 open.

Thank you.
INVITED SPEAKERS

Tomoyuki TSUDA, D.V.M., Ph.D.
Director General
National Institute of Animal Health
National Agriculture and Food Research Organization
JAPAN

Topic: Thailand - Japan Animal Health Research

Naoaki MISAWA, D.V.M., Ph.D.
Director of Center for Animal Disease Control
University of Miyazaki
JAPAN

Topic: The Global Harmonization for Animal Epidemic Prevention Strategies: Approaches for One world, One health

Thanawat TIENSIN, D.V.M., Ph.D.
International Trade and Regulations Group
Division of International Livestock Cooperation
Department of Livestock Development
THAILAND

Topic: Transboundary Animal Diseases and Food Safety Issues: Challenges and Opportunities for Capacity Building of Veterinary Services and Research
Thailand and Japan had more than 50 year history on animal health research. In this presentation, the relationship between our institutions and future directions of collaborative research between Thailand and Japan is focused. The outline of the history is summarized in the table below.

The collaboration was started by control of rinderpest (RP) for development of the livestock industry in Thailand in 1958. The individual technical support for the RP vaccine production was performed for 9 years and was followed by the technical collaboration on FMD for 10 years. This period might be thought as the 1st stage.

In the 2nd stage, two major technical cooperation projects were carried out in two sites, i.e., Packchong and Bangkok. The new buildings and facilities were constructed under the Japanese Official Development Assistance (ODA) in both sites, prior to the projects. These technical cooperation and following JICA projects were continued from 1977 to 2006.

The joint conference was started 2 years ago and Zoonotic Diseases Collaboration Center (ZDCC) was started in 2005. These projects have been executed under the Memorandum of Understanding (MOU) program. This is the latest stage of collaboration.

Table. Technical and Research Collaboration on Animal Health over Fifty years

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<th>Year</th>
<th>Activity</th>
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<tr>
<td>1958-1967</td>
<td>Manufacture of rinderpest vaccine</td>
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<td>1967-1977</td>
<td>Overseas joint research on foot-and mouth disease</td>
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<tr>
<td>1977-1986</td>
<td>Project on animal health improvement program (JICA)</td>
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<td>1986-1993</td>
<td>Establishment project of the National Animal Health and Production Institute (NAHPI) (JICA)</td>
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<tr>
<td>1993-2001</td>
<td>National Institute of Animal Health (NIAH) Project Phase II (JICA)</td>
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<td>2001-2006</td>
<td>Japan-Thailand Technical Cooperation project for Animal Disease  Control in Thailand and Neighboring Countries (JICA)</td>
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<tr>
<td>2005-Present</td>
<td>Thailand - Japan Zoonotic Diseases Collaboration Center (ZDCC)</td>
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<tr>
<td>2012-Present</td>
<td>Thailand - Japan joint conference</td>
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The more stable relationship and collaboration between two countries are expected through above mentioned collaboration projects.

The future directions and the objectives of the research collaboration on animal health would be thought as follows. Namely, to improve the productivity of livestock industry, to ensure the safety and quality of animal products, to provide against the outbreak of the emerging and reemerging infectious diseases, and to contribute to the international one-health policy.

I hope our collaboration will continue producing the fruitful results in future.
The University of Miyazaki is located in southern Kyusyu Island as one of the leading livestock production base in Japan. In 2010, the Graduate School of Medicine and Veterinary Medicine, in which the medicine and veterinary science are integrated, was established as the first graduate school of its kind in Japan. While we have been focusing on the establishment of an education system for infectious diseases, we were confronted with outbreaks of the foot-and-mouth disease in Miyazaki-prefecture in 2010 and about 280,000 cattle and pigs were sacrificed. Fortunately, the disease was contained only in Miyazaki-prefecture and we successfully blocked the disease from expanding to other prefectures. From the experience of the outbreak that caused a huge economic loss, we re-realized the importance of operating early detection and proper preventive measures for trans-boundary malignant contagious diseases in domestic animals.

Based on this experience, the Center for Animal Disease Control (CADIC) was set up in the University of Miyazaki in 2011 October focusing on establishing a world-class study organization. Then, the results of educational activities on the control of foot-and-mouth disease and organization of international symposiums were highly praised as noteworthy initiatives at the National University Evaluation in 2011.

In 2012, we accepted veterinarians from other countries, including ASEAN countries, involved in countermeasures for animal infectious diseases as trainees (JICA issue-specific training: Advanced Education Courses on foot-and-mouth disease). We also conducted the 2nd and 3rd International Symposiums and various workshops and public lectures on topics such as cattle handling and have made available various programs linked to regional and international contributions.

In recent years, the risks of infectious diseases so called “imported infectious diseases” that were previously considered to be irrelevant in Japan have increased with the increase in interactions among people and international exchange of goods. Considering this increase in international movement of people and goods because of globalization, it is difficult to prevent the entry of infectious disease pathogens. Therefore, it is important to keep employing risk management measures in response to these risks. Recently, there have been outbreaks of the H7N9 avian influenza in China, and human cases of infection resulting in death were reported, regardless of its low pathogenicity in birds.

Based on cases of foot-and-mouth disease and highly pathogenic avian influenza experienced in the Miyazaki-prefecture, we have learned that safe and stable livestock management requires daily health management of livestock and disease control activities. Disease control, which requires a significant amount of effort and money, is an activity of perseverance. If no events occur for a certain amount of time, it would seem that the money poured into disease control has been wasted. However, although infectious disease outbreaks can occur despite best efforts, preparation can minimize the number of victims by providing appropriate initial response.

At the CADIC, we have established an advanced educational system for animal disease control through collaboration between the industry, government, and academia utilizing the field of livestock in the Miyazaki-prefecture. In addition, we undertake activities to tackle research topics associated with disease prevention to ensure safety and reliability. We hope to form an international education and research center for animal disease control, and we thank you for your cooperation, guidance, and encouragement.
Transboundary Animal Diseases and Food Safety Issues: Challenges and opportunities for capacity building of veterinary services and research

Dr. Thanawat TIENSIN

Introduction: All animal diseases have the potential to adversely affect human populations by reducing the quantity and quality of food, other livestock products and animal power that can be obtained from a given quantity of resources and by reducing people’s assets (Schlundt et al., 2004). TADs have the potential to threaten food security through serious loss of animal protein and/or loss of draught animal power for cropping; increase poverty levels particularly in poor communities that have a high dependence on livestock farming for sustenance; cause major production losses for livestock products such as meat; milk and other dairy products; wool and other fibers and skins and hides, thereby reducing farm incomes (Otte, 2007). Their occurrence may thereby cause major losses in national export income in significant livestock-producing countries; cause public health consequences in the case of those TADs which can be transmitted to humans (i.e. zoonoses); cause environmental consequences through die-offs in wildlife populations in some cases, and cause pain and suffering for affected animals (Basuno, 2010).

Food production, processing, and marketing systems are complex. In many developing countries they are also highly fragmented and dependent upon a large number of small producers. While this may have socioeconomic benefits, as large quantities of food pass through a multitude of food handlers and middlemen, the risk of exposing food to unhygienic environments, contamination and adulteration increases. Problems occur as a result of poor post-harvest handling, processing and storage of food and also due to inadequate facilities and infrastructure such as the absence or shortage of safe water supply, electricity, storage facilities including cold stores, and transport facilities and networks, etc. Strengthening government regulation and enforcement of corporate food producers, and breaking up their stranglehold on the food system, are key steps to improving food safety (Lynch and Silva, 2013).

The development and growth of many countries, as well as the prevention and control of major biological disasters, depend on the performance of their policies and economies on agriculture, animal health and food and this, in turn, directly relates to the quality of their Veterinary Services (VS). Important roles for VS include veterinary public health - including food-borne diseases - and regional and international market access for animals and animal products. Use of the OIE Tool for the Evaluation of Performance of Veterinary Services (OIE PVS Tool) is a key element in the OIE PVS Pathway in establishing their current level of performance, identifying gaps and weaknesses in their ability to comply with OIE international standards, and forming a shared vision with stakeholders including the private sector.

The Department of Livestock Development (DLD) of the Ministry of Agriculture and Cooperatives (MoAC) of Thailand has as one of its priorities, amongst other tasks, to facilitate the ongoing and improved access to international markets for animals and animal products. Therefore, transboundary animal diseases and food issues are challenges and opportunities for capacity building of veterinary services in Thailand and other developing countries in the region. This report aim at describing experiences of Thailand in strengthening their capacities of veterinary services to deal with transboundary animal diseases and food safety issues.

Lessons learned of HPAI H5N1 outbreaks: The outbreaks of HPAI H5N1 virus in Thailand during 2004-2008 had serious consequences for poultry production, human health, social community, and farmers’ livelihoods (Tiensin et al., 2005; Tiensin et al., 2009). Firstly, the Central Region and the lower part of the Northern Region of Thailand can be considered a high risk area of avian influenza infection (Tiensin et al., 2005; Gilbert et al., 2006; Tiensin et al., 2007a). This could be linked to both the high density of poultry population in these regions and in particular the large numbers of free-grazing ducks raised in the rice fields (Gilbert et al., 2007; Tiensin et al., 2009). Secondly, domestic ducks, excreting HPAI H5N1 virus without signs of illness, hampered detection and control of the disease in 2004-2005 (Sturm-Ramirez et al., 2005; Songserm et al., 2006). Therefore, it is imperative that intensive surveillance programs implemented in these areas were focused to detect avian influenza viruses in duck flocks. Lastly, the comparison between the 2004 and the 2005 episodes suggested that HPAI H5N1 incidence in Thailand was reduced dramatically since stringent control measures had been implemented (Tiensin et al., 2007a).

Most H5N1 infections were observed in backyard poultry in 2004-2005 (Tiensin et al., 2007a). Backyard poultry greatly contribute to local consumption and subsistence agriculture in rural areas. We found that the high density of poultry, the local geography (e.g., wetlands, water reservoirs, and rice paddies), and farming practice in these regions were risk factors for outbreaks. In addition, a strong association between free-grazing duck populations and the practice of free-grazing farming with spread of the virus (Tiensin et al., 2005; Gilbert et al., 2006; Songserm et al., 2006; Tiensin et al., 2009). To elucidate the complex and multi-factorial origins of H5N1 epidemics, a multiple potential pathway was drawn. Figure 1 illustrates a scheme of multiple potential pathways for exposure to and transfer of pathogens in small-scale poultry operations in rural areas (Tiensin et al., 2009). Poultry slaughter houses, fighting cocks, quail flocks, vaccination status, and climate (specifically annual precipitation) have also been found to be associated with outbreaks in poultry. These factors could be potential risk factors for the spread and maintenance
of HPAI virus in Asian countries including Thailand (Kung et al., 2007; Biswas et al., 2008; Fang et al., 2008; Biswas et al., 2009; Minh et al., 2009; Ahmed et al., 2010; Yupiana et al., 2010). Monitoring and surveillance programme in free-gazing ducks and scavenging chickens must be strengthened.

**Animal-human-ecosystem interface and HPAI H5N1**: Understanding the animal-human-ecosystem interface is a critical element in preventing, evaluating and predicting risks of emerging zoonotic diseases, as well as in designing evidence-based programs for prevention and early detection of emerging infectious diseases, such as avian influenza. Poultry production has changed from small-scale methods to industrial-scale operations. There is substantial evidence of pathogen movement between and among these industrial facilities, release to the external environment, and exposure to farm workers, which challenges the assumption that modern poultry production is more biosecure and biocontained as compared with backyard or small holder operations in preventing introduction and release of pathogens (Schlundt et al., 2004; Karesh et al., 2005). The changes in organization, intensity, housing, and waste management may influence the emergence and transfers of avian influenza virus among wild and domestic species, and from avians to human populations. Most importantly, the modern methods of poultry production have changed particularly broiler and layer chickens. These operations result in high numbers of poultry housed under confined conditions at great density and geographic concentration. These methods of food animal production generate many routes of pathogen transfer among wild and domesticated species and from animals to humans through occupational, peri-occupational, and environmental pathways. Figure 2 shows a schematic representation of multiple potential pathways for exposure to and transfer of pathogens within the environment of concentrated animal feeding operations (CAFOs) (Graham et al., 2008; Van Kerkhove et al., 2011). In addition, monitoring poultry population may improve detection of early events in emergence of avian influenza. A careful evaluation of operations at all poultry facilities - large and small - should be undertaken to reduce opportunities for the transmission of disease among avian and other species. Moreover, if appropriate protections such as vaccination are identified, the agricultural workforce constitutes a high-risk population for whom protection from zoonotic disease is important not only for their health but for the health of their communities and the population at large. Finally, improved oversight and management of animal wastes - including transport and sale as well as use in aquaculture or in crop activities - should be included in strategies to reduce risks of pandemic HPAI.

**Key findings of the PVS evaluation**: Thailand has a very strong veterinary services (VS), led by the high standards required of its export industries (e.g. poultry to EU), which has tended to also improve other areas and promote awareness of high quality systems both within DLD and amongst stakeholders, particularly in relation to food safety and traceability systems. Initial and continuing education is very strong across the board. HPAI outbreaks and ensuing political support have also assisted in the rapid high quality development of the VS. Areas such as resourcing, emergency response, laboratories, internal and external coordination are excellent and have been honed through the real experience of dealing with emergency HPAI outbreaks from 2004-2008. GAP and GMP systems are very appropriate ways to improve animal health/food safety measures nationally, and require expansion.
Professional and technical staffing of the veterinary services: The Thai VS are well staffed with veterinarians, except at field level. Qualified veterinarians occupy the majority of positions at DLD central, regional and provincial levels, and in veterinary laboratories. A small minority of district livestock offices are currently led by a veterinarian. Generally, field staff in district, sub-district and village levels and in the majority of border checkpoints and small slaughterhouses are not veterinarians. A large proportion of veterinarians in Thailand are engaged in predominantly small animal practice (> 2000) and do not engage in official activities except small animal surveillance reporting. The Good Agricultural Practice (GAP) and Good Manufacturing Practices (GMP) accreditation systems managed by DLD require services by qualified, approved and trained veterinarians at participating farms, slaughterhouses, milk processors etc. Though these systems ensure high quality veterinary practices, they only cover a small proportion of farms or plants in Thailand, and almost no smaller farms or operations (e.g. smallholders).

Veterinary laboratory diagnosis and quality assurance: Thailand’s laboratory diagnostic services are of high quality, have high capacity and are well managed. All official diagnostic tests are performed at DLD laboratories and the lab network is well coordinated with standardised testing practices across the country, facilitated through consistent lab standard operating protocols and nationally coordinated proficiency testing. Thailand has the designated OIE Regional Reference laboratory for FMD (South-East Asia), and the ASEAN Food Reference Laboratory for Veterinary Drug Residues, and international collaboration with global networks are strong. NIAH has a special Mobile Rapid Response Team and accompanying vehicle with emergency diagnostic equipment.

Epidemiological surveillance: Animal health surveillance in Thailand is strong for GAP certified farms and for targeted poultry diseases. There is a strong disease reporting system from district to central levels although only the poultry version is currently web-based. There is good capacity for disease investigation and sampling at district and provincial levels. Gaps exist in the field in relation to smaller, non-poultry farms relating to the skills and coverage of Thailand’s field animal health surveillance network.

Food safety and veterinary drug controls: High food safety standards exist with veterinary ante and post mortem inspection and laboratory testing facilities at all export premises and for GMP certified domestic slaughterhouse and processing operators. Lesser standards apply for the remainder of operators servicing the domestic market, with lower standards applying based on the decreasing size of the operation. Efforts are being applied to improve standards through expanding GMP certification and otherwise raising standards and requirements for smaller operators such as through training of non-veterinary meat inspectors. At the retail level DLD is piloting a hygienic meat project which is showing good promise to improve safety standards at retail level with financial benefits to owners who undertake such an investment.

The regulation of veterinary medicines and biologicals is a significant gap for the Thai VS, who do not have adequate direct involvement, nor collaboration and/or technical influence with the relevant authority in FDA. DLD only has direct authority over regulation of the category of hazardous substances (disinfectants, pesticides etc).

Major gaps of Thai Veterinary Services: Major gaps identified are a lack of field veterinarians and a non-standardised para-professional category. The lack of regulatory control over drug sales and their use means there is no effective or skilled field animal health network (outside of GAP) with impacts negatively on passive surveillance/early detection, disease control and public-private partnerships (no private field vets, as they cannot make an income from farmers without controlling drug sales). Livestock assistants and volunteers are too poorly trained and are basically outposts for reporting what they “notice”. However, poultry surveillance is much stronger as it counts with x-ray surveillance, GAP etc. There is also a lack of cost recovery, such as in laboratory testing or in the implementation of GAP/GMP. Smallholders need greater representation and consultation generally. Domestic food safety, especially in smaller slaughtering establishments, milk collecting centres etc. still needs attention in order to guarantee the same high quality food to Thai people as is provided for exports. There is still room for improvement in technical independence in some highlighted areas like the disinfection in villages and wild bird habitats, border closure and private clinic accreditation (Hutter, et al., 2012)

Conclusions: The Veterinary Services of developing countries are in urgent need of support to provide them with the necessary infrastructure, resources and capacities that will enable their countries to benefit more fully from the WTO Sanitary and Phytosanitary Agreement (SPS Agreement) while at the same time providing greater protection for animal health and public health and reducing the threat for other countries which are free of diseases. It is considered the Veterinary Services as a Global Public Good and their bringing into line with international standards (structure, organisation, resources, capacities, role of paraprofessionals) as a public investment priority. Transboundary Animal Diseases and Food Safety Issues are our challenges and opportunities for capacity building of veterinary services and resea to seek for further insight information to deal with these problems.
- Experimental Infection of Animals with a Foot-and-Mouth Disease Virus Isolated from the 2010 Epidemic in Japan
- Molecular Epidemiology Surveillance of Foot and Mouth Disease Virus type A in Thailand during 2012-2014
- Transition of Bovine Viral Diarrhea Virus Subgenotypes in Japan
- Detection of Bovine Leukemia Virus Proviral DNA Associated with Lymphosarcoma by In situ Hybridization Technique
- Avian Paramyxoviruses are Vaccine Vector Candidates that Escape Immunity Conferred by NDV Vaccination in Chickens
- Genetic Variation of Infectious Bronchitis Virus in Southern Thailand
- Genetic Analysis of an H5N8 Highly Pathogenic Avian Influenza Virus Isolated from Chickens in Japan in 2014
- Application of the PigINFO Benchmarking System to Analyze Effects of PRRS Virus on Herd Productivity
- Pathogenicity of Porcine Reproductive and Respiratory Syndrome Viruses Isolated in Japan
- A Case Report: Orf Virus in Goats in Chonburi Province
- Multilocus Sequence Typing Analysis of Melissococcus plutonius Isolated from European and Japanese Honeybees: Spread of some Sequence Types Across Borders and Bee Species
- Characterization of Virulence-Resistance Plasmids Carried by Emerging Multidrug Resistant Salmonella Typhimurium Isolated from Cattle in Hokkaido, Japan
- Mapping of Q fever in Ruminants, Thailand during 2012-2013
- Role of Cell-Surface Molecules of Erysipelothrix rhusiopathiae in Adherence to Porcine Endothelial Cells
- Development of Novel PCR-based Method for Typing of Capsular Polysaccharide Synthesis Gene Clusters of Streptococcus suis
- Seroprevalence and Risk Factors of Toxoplasma gondii Infection in Goats and Sheep in the Western Thailand
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- A Surveillance of Pesticides Contamination in Pineapple Peel Using for Dairy Cattle Feed

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- Case Report: Co-incidence of Jaagsiekte Sheep Retrovirus and *Manheimia haemolytica* Infections in a Sheep Farm at Phitsanulok Province

- Comparison of Serological Tests for Antibody Detection Against *Brucella abortus* Infection in Cattle and Buffaloes

- A Serological Survey of Classical Swine Fever in the Eastern Region of Thailand

- Development of Method for Carbamate and Organophosphate Poisoning Diagnosis in Livestock Samples by pH meter

- Development of the Rapid Immunological Methods for Diagnosis of Leptospirosis in Livestock

- Sequencing analysis of Elephant Endotheliotropic Herpes Virus (EEHV) Obtained from Dead and Live Asian Elephants in Thailand

- Genetic Analysis of Cricket Paralysis Virus: The Emerging Disease of House Cricket in Thailand

- Seroprevalence of Paratuberculosis in Dairy Cattle in the Upper Northeastern Region of Thailand by Complement Fixation Test and Fecal Smear

- Monitoring of Chicken Infectious Anemia in the Risked Broiler Farms


- From the Past to Present: Electronmicrograph Demonstration of Veterinary Pathogenic Agent Isolation in Pathology Section, NIAH, Thailand
Experimental Infection of Animals with a Foot-and-mouth disease virus isolated from the 2010 epidemic in Japan

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Introduction: Foot-and-mouth disease (FMD) is a highly contagious disease of cloven-hoofed animals and occurs endemically or sporadically in many countries. At present, Japan is an FMD-free country where vaccination is not practiced; however, FMD occurred in Japan in 2010. In the epidemic, many FMD viruses (FMDV) were isolated from collected clinical samples. To understand the pathogenesis of the FMDV isolates will be useful to develop control strategies in future epidemics elsewhere. Therefore, several experimental infections of animals with the isolate have been performed in our institute.

Methods: The FMDV O/JPN/2010 used in this study was isolated from the first case using primary bovine kidney cells and passed twice in BHK-21 cells. Cattle, goats and pigs were inoculated with $10^6$ TCID$_{50}$ of the isolate by an intradermal route and animals of the same species were cohabited with the inoculated animals. Pigs were also inoculated with $10^2$ or $10^6$ TCID$_{50}$ of the isolate by intranasal and intraoral routes. Clinical signs were observed daily. Clinical samples were collected routinely. Virus isolation and titration were performed using IB-RS-2, ZZ-R 127 and LFBK-αβ cells. Detection and quantification of viral genes were performed by an RT-PCR assay and a real-time RT-PCR assay. Antibody titers were determined by a neutralization test and a liquid-phase blocking ELISA.

Results: Vesicular lesions were developed in the cattle and pigs inoculated by the intradermal route and in the contact animals. Vesicular lesions of the pigs were more severe than those of the cattle. On the other hand, the inoculated and contact goats showed mild vesicular lesions. Although clinical signs were observed in the pigs inoculated with $10^6$ TCID$_{50}$ of the isolate by the intranasal route, they were not observed in the pigs inoculated with $10^3$ TCID$_{50}$ of the isolate by the same route. In contrast, clinical signs were observed in all the pigs inoculated by the intraoral route; however, the pigs inoculated with low dose of the isolate showed them later than the pigs inoculated with high dose of the isolate. Viruses and viral genes were detected from the clinical samples collected from all the inoculated and contact animals except for the pigs inoculated with $10^3$ TCID$_{50}$ of the isolate by the intranasal route. Similarly, antibody responses were confirmed in all the inoculated and contact animals except for the pigs inoculated with $10^3$ TCID$_{50}$ of the isolate by the intranasal route.

Conclusions: The experimental infections showed that the isolate caused the synchronous disease in the animals inoculated with the isolate by the intradermal route and efficient spread to the contact animals. Dose-dependent responses were also confirmed in the pigs inoculated by both intranasal and intraoral routes and minimal effective infectious dose by intraoral inoculation seems to be lower than that by intranasal inoculation. These results provide a better understanding of the nature of FMD in susceptible animals.

Keywords: cattle, experimental infection, foot-and-mouth disease virus, goat, pig
Molecular Epidemiology Surveillance of Foot and Mouth Disease Virus type A in Thailand during 2012-2014

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Introduction: Foot and mouth disease (FMD) is a severe, highly contagious viral disease of livestock with significant economic impact. The disease affects cattle and swine as well as sheep, goats and other cloven-hoofed ruminants. The organism which causes FMD is an Aphthovirus of the family Picornaviridae. It consists of a small, non-enveloped capsid and a single strand of positive sense RNA. There are seven serotypes of the virus O, A, Asia1, C, SAT1, SAT2 and SAT3. Infection or vaccination with one serotype does not confer protection against other serotypes (Mattion et al., 2004). In the past few years, FMDV serotype A is considered endemic in Thailand. Therefore in this study, we used the nucleotide sequencing method to investigate the genetic characterization of foot and mouth disease virus serotype A in Thailand during 2012-2014. The molecular epidemiological information in this study is benefit for database, investigation of genetic variables and support the seed virus selection to enhance the efficacy of vaccine production.

Materials and Methods:
Viruses: Twenty seven samples of FMDV serotype A in Thailand during 2012-2014 were conducted by ELISA typing (Roeder and LeBlanc Smith, 1987).
RNA: Total RNA was extracted from epithelial suspension or cell culture by using Trizol® LS reagent (Invitrogen™, USA) and re-suspended in nuclease-free water.
Reverse Transcription (RT): The single-stranded RNA is reverse transcribed into complementary DNA by using a reverse transcriptase enzyme, following thermal profile was used: 42°C for 60 min and 95°C for 5 min. The newly synthesized cDNA was amplified using for the template in the Polymerase Chain Reaction.
Polymerase Chain Reaction (PCR): cDNA was used as the template in the PCR. The following thermal profile was used: 94°C for 4 min; 30 cycles of 94°C for 60 sec., 55°C for 60 sec. and 72°C for 90 sec; followed by a final extension of 72°C for 5 min. PCR products were analysed by electrophoresis. Post-PCR removal of deoxynucleoside triphosphates and primers were achieved using the QIAquick PCR Purification Kit (QIAGEN, Germany) and resuspended in nuclease-free water. RT-PCR was performed using foot and mouth disease virus (FMDV) specific primers 1C-612 and NK61 (Knowles and Samuel, 1998) targeting regions either side of the VP1 gene (Table 1).
Sequencing: PCR amplicons were sequenced by using the Big-Dye® terminator cycle kit version 3.1 (Applied Biosystems, USA), with the 1C-612 and NK-72 sequencing primers (Table 1). The sequencing reactions were run on an automated sequencer (ABI 3130 Genetic Analyzer).
Phylogenetic analysis: A Neighbor-joining (unrooted trees) was constructed by using MEGA version 4 (Tamura, et al., 2007). The robustness of the tree topology was implemented in the program.

Table 1 Oligonucleotide primers used for RT-PCR and sequencing of FMDV serotype A.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer sequence (5'-3')</th>
<th>Gene</th>
<th>Product length</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>1C-612</td>
<td>TAGCGCCGGCAAGACCTTGA</td>
<td>1C</td>
<td>813 bp</td>
<td>RT-PCR, Sequencing</td>
</tr>
<tr>
<td>NK72</td>
<td>GAAGGGCCCAGGGTGGACTC</td>
<td>2A/2B</td>
<td>Universal primer</td>
<td>Sequencing</td>
</tr>
<tr>
<td>NK61</td>
<td>GACATGTCCTCCTGCACTG</td>
<td>2B</td>
<td>Universal primer</td>
<td>RT-PCR</td>
</tr>
</tbody>
</table>

Results: Twenty seven samples of foot and mouth disease virus serotype A in Thailand during 2012-2014 were performed by ELISA typing and PCR technique using specific primer 1C-612/NK61. The PCR product (813 bp) analysis was performed using nucleotide sequencing. The complete sequence of 636 nucleotides VP1 genome from isolates of serotype A were compared and analyzed as phylogenetic tree. It was found that all of FMDV serotype A did not have genetic variables and belonged to ASIA topotype (Fig 1).

Conclusions: Foot and mouth disease virus serotype A in Thailand during 2012-2014 did not have genetic variable and belonged to ASIA topotype. The information in this study can be used to support the seed virus selection to enhance the efficacy of vaccine production.

Keywords: Foot and mouth disease virus, serotype A, nucleotide sequencing, topotype
Fig 1  Phylogenetic tree of FMDV serotype A in Thailand during 2012-2014 (underline)

Acknowledgement: We would like to thank RRL staffs for their help and participation in this successful study.

References


Transition of *Bovine viral Diarrhea Virus* Subgenotypes in Japan

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**Introduction:** *Bovine viral diarrhea virus* (BVDV) is classified into the genus *Pestivirus* in the family *Flaviviridae*. BVDV is widely distributed throughout the world and causes a chronic wasting disease, bovine viral diarrhea (BVD), of cattle. BVDV strains isolated to date are genetically and serologically divided into two genotypes and more than ten subgenotypes (1).

To utilize for preventive measure of BVD in Japan, genetic analysis of Japanese isolates was carried out in the present study.

**Methods:** One hundred and fifty-five of BVDV isolates are collected from 17 prefectures in 2006–2013 in Japan. The 5'-untranslated region of the isolates was amplified by reverse transcription-polymerase chain reaction (2). Subsequently, the nucleotide sequence of the amplified fragments was determined using automatic DNA sequences. The phylogenetic tree was constructed by the neighbor-joining method and the subgenotypes of the Japanese isolates were identified.

**Results:** Japanese isolates of BVDV were segregated into four subgenotypes: 35 isolates (22.6%) of 1a, 58 isolates (37.4%) of 1b, 13 isolates (8.4%) of 1c, and 49 isolates (31.6%) of 2a. The most common subgenotypes isolated each year were 1a in 2006 and 2008, 1b in 2007 and 2009, 2a in 2011 and 2012. In 2010 and 2013, both of 1b and 2a were predominant.

**Summary/Conclusions:** In the present study, 155 of Japanese BVDV isolates were classified into four subgenotypes. The population of BVDV belongs to subgenotypes 1a and 1c seems to decrease year by year. On the other hand, the percentage of subtypes 2a in all isolates was increased from 2010 and keep high values until 2013. Subgenotype 1b was isolated constantly throughout the investigation period.

These results suggest that the infection caused by subgenotypes 1b and 2a are prevalent in recent years in Japan. Therefore, it should be important to implement preventive measures against both of these two subgenotypes.

**Keywords:** Pestivirus, Bovine viral diarrhea virus, Phylogenetic analysis, Subgenotype, Japanese isolate

**References**


Detection of Bovine Leukemia Virus Proviral DNA Associated with Lymphosarcoma by In situ Hybridization Technique

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Introduction: Generally the most common neoplasm in cattle can be divided into 2 types, Sporadic bovine leukosis (SBL) and Enzootic bovine leukosis (EBL). SBL caused by unknown etiology, can be subdivided into 3 subtypes: calf, juvenile and cutaneous form. Age and clinical manifestations of these subtypes are varied which can be occurred from 1.6 months to 3 years of age, and the affected organs mostly are thymus and lymph nodes (Reed, 1981). EBL caused by bovine leukemia virus, Oncogenic Lymphotropic Retrovirus. The virus infects B lymphocytes (Gillet et al., 2007), and induces tumor formation in B cell lineage. Abnormal lymphocytes can be seen from blood smear as well (Shigeru et. al., 1993). Moreover, animals are undergone life-long infection because Proviral DNA of the virus can integrate into host genomic DNA. The aim of this study is to perform in situ hybridization technique developed to increase sensitivity and demonstrate Proviral DNA in an animal found death from lymphosarcoma.

Materials and Methods: Necropsy and Histopathology: A death dairy cattle was brought to necropsy in pathology section, National Institute of Animal Health. Various organs were collected to investigate microscopically. Fixed organs were made histopathological slides according to Luna (1968).

Probe production by nested PCR: Template DNA was used from plasmid harbored env gene of EBL showing leukemia from blood smear characterized by neoblastic lymphocyte (Fig.3). Nested PCR developed by Fechner et al., 1996 was performed to amplify specific target DNA and produce probe. The hybridization probe was produced from the second PCR by mixed dNTP and Biotin-dUTP (Jena Bioscience, Germany).

In situ hybridization (ISH): Paraffin embedding blocks were cut into 3 µm and put on positive glass slides. Deparaffinization, rehydration, and unmask were performed. Probe concentration of 500 ng/slide was used. Prehybridization and hybridization were done in Hybridizer according to manufacturers instruction (DAKO®) for 16 hr. Color was developed by DAB chromogen (DAKO®). Finally slides were counter stained with hematoxylin.

Results: Necropsy and Microscopic finding: Abnormal masses at various sizes were found at right atrium omentum and lymph node (Fig.1). Neoplastic cells microscopically were found vesicular nucleus and mitotic figure of nuclear chromatin proliferation (Fig.2).

PCR analysis and Probe production: PCR product and In situ hybridization probe from biotin labelling were successfully amplified with expected size approximately 444 bp (Fig.4).

In situ hybridization (ISH): BLV infected cells were detected by ISH technique in nucleus and cytoplasm showing brown pigment (Fig.5).

Conclusion: To our knowledge, this is the first report to be able to detect Proviral DNA in lymphosarcoma by ISH in naturally infected animal. Moreover, this study gives the same result found by in situ-PCR as the study of Xie et al., (1997). This technique is highly sensitive due to repeating the step of streptavidin (Dako™) during the process of detection. Additionally, this technique is established in order to be used for differentiation microscopically between SBL and EBL in affected animals from lymphosarcoma.

Keywords: Enzootic bovine leukosis, In situ hybridization technique, diary cattle

Acknowledgements We would like to express our sincere gratitude to Dr. Chira Kongkrong, Dr. Ladda Trongwongsa and Dr. Monaya Ekgatat for their valuable advice, knowledge transfer and facilitation in the study. Additionally we would like to thank our pathology colleagues for their determination effort and assistance.
**Fig 1** Tumor mass presents at right atrium (A), cardiac muscle, loss of striation (B), Abnormal mass of lymph nodes at omentum (C), enlargement of left prefemoral lymph node (D).

**Fig 2** Proliferation of tumor cell in uterus (A), lymph node (B and D), cardiac muscle (C).

**Fig 3** Abnormal lymphoid cells appeared in peripheral blood.

**Fig 4** The amplified env (598 and 444 bp) containing 6x loading buffer, Novel juice® were visualized using UV light. Lane M: 100 bp DNA ladder; lane 1 is plasmid harbored env gene; lane 2 is hybridization probe, lane 3 is negative control.

**Fig 5** Positive brown pigment labelling is observed in nucleus and cytoplasm of infected lymphocytes in lymph node.

**References**


Avian Paramyxoviruses are Vaccine Vector Candidates that Escape Immunity Conferred by NDV Vaccination in Chickens
Avian paramyxoviruses evading immunity against Newcastle disease virus

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Background: Various viruses are used as recombinant vaccine vectors to prevent infectious diseases in poultry. Newcastle disease virus (NDV), also known as avian paramyxovirus (APMV) serotype 1, has been used as a vaccine vector to express the hemagglutinin (HA) protein of avian influenza virus (AIV). To control a severe avian influenza (AI) outbreak in chickens, however, a live NDV recombinant vaccine expressing AIV HA is not suitable in emergency vaccination programs because commercial chickens often possess pre-existing immunity to NDV induced by routine vaccination. Therefore, a novel vaccine vector is required for emergency vaccination of chickens against AI. APMVs of serotypes other than serotype 1 also replicate in mucosal tissues where AI viruses primarily replicate, and scarcely cause diseases in chickens. We investigated whether APMV strains of other serotypes could be used as vaccine vectors that could evade pre-existing immunity acquired by NDV vaccination in chickens.

Methods: To evaluate the effect of NDV vaccination on replication of APMVs, the cross-reactivity of anti-NDV sera with APMV serotype 2 to 10 was examined by hemagglutination inhibition (HI) and virus neutralization (VN) tests. Anti-NDV sera used for serological tests were collected from chickens at seven weeks of age, which were inoculated with a live NDV at two and four weeks and with an inactivated NDV at six weeks. In addition, APMVs were inoculated in naive or NDV vaccinated seven-week-old chickens and virus shedding, induction of the specific antibody response and pathogenicity in chickens were examined.

Results: When compared with the other serotypes of APMV, APMV serotype 2, 6 and 10 were less cross-reactive with antibodies against NDV in HI and VN tests. Antibody responses were induced by oculo-nasal inoculation with APMVs of serotype 2, 6 or 10 in naive chickens. Antibody responses were also induced by these APMV in chickens that were prior-vaccinated twice with NDV. Viral shedding was limited in the respiratory tract and not observed in the cloaca. None of chickens showed any clinical symptoms during the observational period.

Conclusion: These results showed the potential of APMV serotype 2, 6 and 10 as vaccine vectors for emergency vaccination against AI in chickens. Especially, APMV serotype 10 is the best vaccine vector candidate for use in conventional chickens because natural infection of APMV serotype10 are unlikely to occur in poultry.

Keywords: Avian paramyxovirus (APMV); Newcastle disease virus (NDV); vaccine vector; antibody response
Introduction: Infectious bronchitis (IB), caused by gammacoronavirus avian infectious bronchitis virus (IBV), is among the major highly contagious diseases of the poultry industry. IB is frequently involved in upper respiratory disease, nephritis and enteritis. In layers, poor fertility and low egg production commonly occurs. Recently, disease appears to extensively affect other tissues e.g. proventriculus (de Wit et al., 2011). The structural glycoprotein spike (S) of IBV is processed into the S1 and the S2 subunits. Function of the S1 protein is to attach host cell receptor molecules and composes of epitopes and determinants for virus neutralizing, protective immunity, and serotype specificity. Thus, the S1 gene analysis has been widely used to differentiate IBV genotypes and serotypes. Genetic mutation in the hypervariable region of the S1 would generate new strains of IBV worldwide (Jackwood et al., 2012). We aim to investigate the IBV situation by collecting the confirmed IBV field isolates from different bird types isolated from different regions in southern Thailand from 2008-2013, differentiating them by sequence analysis and determining their relationships with vaccine and neighboring strains.

Methods: Twenty four samples of IBV isolated in southern Thailand between 2008 and 2013 were propagated by 9-day-old egg inoculation and were characterized by immunofluorescence on infected CAM after 3-5 blind passages. The IBV isolates were further confirmed from harvested allantoic fluid by RT-PCR to target the amplified 3’UTR cDNA fragment screened for all IBV strains. Subsequently, the obtained IBVs were phylogenetically characterized by RT-PCR using the S1 gene primer set (Adzhar et al., 1996; Cavanagh et al., 2002). The purified PCR products were sequenced by commercial service (SolGent ASSA service, Korea). The partial S1 nucleotide and deduced amino acid sequences from the isolates were assembled, and aligned using a BLAST search via NCBI and selected reference IBV vaccine strains. The multiple sequence alignments and determination of the nucleotide and amino acid identities were performed using Clustal W in the Bioedit software version 7.2.5.0. Aligned sequences were phylogenetically analyzed using the neighbor-joining method available in the MEGA software version 6.0 (Tamura et al., 2013). The bootstrap values were determined from 1000 replicates of the original data to designate confidence levels to branches.

Results: The local isolates could be categorized into four groups during six years of investigation ranging from 2008 to 2013. Group I, the unique THA001, containing seven isolates, were clustered between 2008 and 2009. Group II, 15 isolates of QX-like IBV were circulated from 2009 to 2013. Group III a single isolation of a Mass-type vaccine strain was isolated in 2012, and group IV, a virus belonging to an IBV 4/91 strain, was recovered in 2013. Of total, 62.5% of infected birds were village chickens which harbor the major IBV group I and group II. The diseases were associated with non-specific clinical symptoms, exhibiting mild to moderate illness such as depression, respiratory distress and enteric signs. None of these two isolate groups could be matched to the vaccine strains commercially used in Thailand. The group I viruses shared 79-85% both nucleotide and amino acid identity with a Mass-type vaccine strain while the group II viruses yielded low identity values close to a 4/91 strain less than 82.5% nucleotide identity and less than 80.5% amino acid identity. In addition to the minor IBV groups, a virus of group III was isolated from a depressed broiler while an IBV isolate of group IV was isolated from a layer associated with mild moist-rale and low egg production.

Conclusion: IBV remains endemic in southern Thailand. Spread of IBV through the village chicken population within the area is the risk of on-going IBV dissemination. Consequently, IBV investigation in particular village chickens should be regularly monitored. Control strategy in order to decrease IBV infection by arranging a compartment system, biosecurity, vaccine matching and proper vaccination program within the affected area must be applied.

Keywords: Infectious bronchitis virus, S1 glycoprotein, Phylogenetic analysis, Native chickens, Southern Thailand

References
Figure 1 Phylogenetic relationship of Thai IBVs in southern Thailand compared to the reference strains. The isolates sequenced in this study are illustrated with black triangles, black squares, black diamonds and black circles. Open squares indicate the IBV vaccine strains.

Figure 2 Geographic distribution of southern IBV isolates between 2008 and 2013. The symbols are indicated as;

- ▲ = IBV group I 2008 isolates,
- ▼ = IBV group I 2009 isolates,
- ▤ = IBV group II 2009 isolates,
- □ = IBV group II 2010 isolates,
- ▲ = IBV group II 2012 isolates,
- ○ = IBV group IV 2013 isolate.
Genetic Analysis of an H5N8 Highly Pathogenic Avian Influenza Virus Isolated from Chickens in Japan in 2014.

H5N8 influenza virus in Japan in 2014

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Introduction: Increasing mortality rate of chickens of a farm in Kumamoto prefecture Japan was reported in April 2014. H5N8 highly pathogenic avian influenza (HPAI) viruses were isolated from tracheal and cloacal swabs of affected chickens in the farm. An isolate was analyzed genetically and investigated its infectivity for chickens.

Methods: Tracheal and cloacal swabs were obtained from five dead chickens. Pooled tracheal or cloacal swabs were inoculated into allantoamniotic cavity of 10-days-old embryonated eggs. After incubation for 30 hour at 37°C and overnight at 4°C, allantoic fluids of the eggs were collected. The virus isolate contained in allantoic fluid of eggs inoculated with a pooled cloacal swab was designated as A/chicken/kumamoto/1-7/2014 (H5N8), and used for further analyses. Whole genome sequence of the virus was determined by a high-throughput next-generation sequencer and was phylogenetically analyzed by the neighbor-joining method. For assessment of pathogenicity of A/chicken/kumamoto/1-7/2014, 0.2 ml of a 1/10 dilution of the infectious allantoic fluid (64HA) of eggs was intravenously inoculated into eight 5-week-old chickens and lethality was observed for 10 days.

Results: Putative amino acid sequence adjacent to the HA cleavage site had multiple basic amino acids (PLREERRRKR/GLF), which is a signature of high pathogenicity of the virus in chickens. The phylogenetic analysis showed that all eight genomic segments of A/chicken/Kumamoto/1-7/2014 have highest sequence homologies (>99%) with each segment of A/bikal teal/Korea/Donglim3/2014 and A/boriler duck/Korea/Buan2/2014, H5N8 HPAI viruses isolated Korea in 2014. The PB2, HA, NP and NA segments of these viruses were thought to be originated from some H5N8 HPAI virus, such as A/duck/Jiangsu/k1203/2010, which isolated in Jiangsu China in 2010, whereas the PB1, PA, M and NS segments were thought to be derived from some H5N1 virus, such as A/duck/Eastern China/1111/2011 or A/goose/Eastern China/1112/2011. All chickens intravenously inoculated with the virus were dead within 3 days.

Conclusion: All eight genomic segments of A/chicken/Kumamoto/1-7/2014 were very similar to each segments of H5N8 HPAI viruses isolated in Korea shortly before in 2014. Putative amino acid sequence and intravenous inoculation test reveals that A/chicken/Kumamoto/1-7/2014(H5N8) was categorized into HPAI virus.

Keywords: Highly pathogenic avian influenza; H5N8; phylogenetic analysis; infectivity.
Effect of PRRS virus on productivity

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Introduction: Several different data management software packages are currently used among Japanese swine farms, making it difficult to integrate data from different sources, resulting in limited scale epidemiological surveys. Benchmarking systems that continuously compare and measure processes and performance have been used in many areas to evaluate production. We have applied this system to build a data recording system (PigINFO) for evaluating Japanese swine farm productivity. This system was then used to process information on the herd-level infection status of porcine reproductive and respiratory syndrome virus (PRRSV) to evaluate the effects of PRRSV status on herd-level productivity.

Materials and Methods: The study was conducted from January 1 to December 31, 2010. The target population comprised 92 farrow-to-finish pig farms that were clients of consulting veterinarians. Farmers who agreed to participate in this survey were requested to submit farm census data, including average female pig inventory, total numbers of marketed pigs, numbers of deaths at the postweaning stage, total litters, total pigs born alive, total pigs weaned, total pigs bred, and average number of pigs at the postweaning and preweaning stages. From these data, productivity variables, such as postweaning mortality (POWM), preweaning mortality, pigs weaned per mated female per year, pigs born alive, litters per mated female per year, farrowing percentage, and daily weight gain (DWG) were calculated. From each herd, three sets of blood samples were taken from different age groups of growing pigs and sows. These were tested for PRRSV by PCR and ELISA. Herds which were positive by one of these tests were defined as positive. The rest were defined as negative. The effects of PRRSV status on productivity were determined by comparing variable means between positive and negative herds.

Results: A total of 76 herds were PRRSV positive and 16 herds were negative. PRRSV negative herds had 2.2% lower POWM and 53.7 (g/day) higher DWG compared with positive herds (Table 1). None of the other parameters had significant differences when comparing PRRSV status.

Table 1 Comparison of production variables between PRRSV positive and negative herds

<table>
<thead>
<tr>
<th>Production variables</th>
<th>PRRSV positive</th>
<th>PRRSV negative</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postweaning mortality, %</td>
<td>n 6.1</td>
<td>3.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Prewearing mortality, %</td>
<td>n 8.1</td>
<td>3.6</td>
<td>0.22</td>
</tr>
<tr>
<td>Pigs weaned per mated female per year</td>
<td>n 23.2</td>
<td>2.0</td>
<td>0.72</td>
</tr>
<tr>
<td>Pigs born alive</td>
<td>n 10.8</td>
<td>0.6</td>
<td>0.38</td>
</tr>
<tr>
<td>Litters /mated female/ year</td>
<td>n 23.0</td>
<td>2.1</td>
<td>0.45</td>
</tr>
<tr>
<td>Farrowing percentage, %</td>
<td>n 85.6</td>
<td>7.9</td>
<td>0.77</td>
</tr>
<tr>
<td>Daily weight gain (g/day)</td>
<td>n 585.1</td>
<td>48.1</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Conclusions: These results show that PRRSV status has a significant effect on farm productivity. PRRSV caused respiratory syndromes resulting in higher postweaning mortality. PRRSV also decreased growth performance, shown via DWG, suggesting that PRRSV poses a problem even in the absence of clinical signs. No association was identified between PRRSV and reproductive parameters. This may be because of the limited sample size, less virulent virus, or these viruses had less impact on reproductive performance. We are now establishing a large bidirectional network between producers and researchers. Data are obtained quarterly bases, which enables more rapid analysis and allows to provide more timely advice to farmers. Future work will involve examining swine productivity in other countries using the same system. This will allow us to make a more objective comparison of swine productivity between different countries, which may provide insights for future collaborative works in Japan and other countries.

Keywords: Benchmarking, Postweaning mortality, Productivity, PRRS

References
Introduction: Porcine reproductive and respiratory syndrome (PRRS), an infectious disease of pigs, causes reproductive failure in sows and respiratory disorder in young pigs, and is one of the most economically important swine diseases in the pig production countries. PRRS virus (PRRSV) causing this disease is divided into two genotypes, European type and North American (NA) type. Both genotypes PRRSVs are antigenically and genetically diversity. In phylogenetic analysis, ORF5 sequences of NA-type PRRSV strains in Japan were classified into five clusters. The pathogenicity of PRRSV field isolates in each cluster was examined in experimental studies to obtain useful information for control of PRRS.

Methods: Nine Japanese reference strains in each cluster were used in this study; Nagasaki 11-14 (cluster I), P192-5 and P209-13 (cluster II), EDRD1, Yamagata 10-7, Saga 09-3 and P202-1 (cluster III), Jpn5-37 (cluster IV), Jos1 (cluster V). Five 4- to 5-week-old pigs were used in the viral infection experiment for each strain, and all animals were free of PRRSV and porcine circovirus 2 infection. Pigs were intranasally inoculated at the dose of $10^5$ TCID$_{50}$/pig PRRSV. Pigs were monitored for clinical signs and rectal temperature every day. Serum was sequentially collected for the amount of viral RNA measured by a quantitative real-time RT-PCR. The postmortem examination was performed at 21 or 22 day post-inoculation (dpi). The tonsil, lung and lymph node were collected for histopathology and the measurement of viral load.

Results: A fever (>39.5 °C) was observed in pigs inoculated with each strain after 2 dpi, but no fever in Yamagata 10-7. Other clinical sings of all pigs were not observed during an experimental period. The viral RNA in serum from pigs inoculated with each PRRSV strain was detected from 1 or 2 dpi until postmortem day (Fig. 1). The peak of viral RNA (approximately $10^5$ TCID$_{50}$/ml) in serum from pigs inoculated with P192-5, P209-13, EDRD1, P202-1, Jpn5-37 and Jos1 was detected at 5, 7 or 10 dpi and was decreased after the peak. The peak of viral RNA in serum from pigs inoculated with Nagasaki 11-14 was observed at 16 dpi and the level of that, approximately $10^4.5$ TCID$_{50}$/ml, persisted from 5 to 16 dpi (Fig. 1A). The viral RNA from pigs in Yamagata 10-7 and Saga 09-3 persisted between $10^{1.53}$ to $10^{3.1}$ TCID$_{50}$/ml from 1 to 21 dpi (Fig. 1B). In postmortem examination, no clear pathologic lesions were identified in the inoculated pigs. Slightly or moderately interstitial pneumonia was observed in the inoculated pigs by histopathology, and PRRSV antigen was detected in the lungs of some inoculated pigs by the immunohistochemistry.

Conclusions: The viral RNA in serum from pigs inoculated with two cluster III PRRSV strains, EDRD1 and P202-1, cluster II and IV strains, had approximately 10 to 100-fold difference compared with those from other cluster strains. The propagation of virus in pigs differed among PRRSV strains. The transient fever was observed in pigs inoculated with each Cluster virus strain, but the clinical signs such as respiratory disorder were not found. The Japanese PRRSV field isolates caused the low pathogenic disease in the viral infection experiment. Therefore, serious clinical signs might be caused by the infection with other pathogens in field.

Keywords: PRRS, North American type, pathogenicity, cluster
Introduction: Orf (contagious ecthyma or scabby mouth) is a skin disease affecting domestic goats, sheep and cattle. An additional hazard is zoonotic. It is caused by orf virus genus parapoxvirus of the family poxviridae. Most of lesions are found generally on the mouth and face and they are also noticed on the feet of infected animals. The initial signs are papules, pustules and vesicles observed on the lips, nose, ears and/or eyelids and sometimes on the feet or perineal region. Animal repeatedly infected show smaller and early recovered lesions than animal undergone the primary infection (Zhang et al., 2010). In April 2013, 4 scab samples were collected from lesions of clinically suspected goats in a farm located in Chonburi province. 60 goats (4 bucks, 22 does and 34 kids) were raised in this farm that there were 12 affected kids showing small vesicles and scabs on their lips, nose and feet (Figure 1). 8 out of 12 kids died 7-8 days after onset of illness.

Methods: Based on molecular biology diagnosis technique, real time (RT) and duplex PCR were used for viral identification. Orf virus was detected specifically by RT-PCR and they were distinguishable from simultaneously co-infected capripox virus. Fort the real-time PCR reaction, forward primer: 5’-TACACGGAGTTGCGCCGTGATCTTGTA-3’, reverse primer: 5’-CGCCAAGTACAAGAAGCTGATGA-3’ and probe sequence: 5’Hex-TG-CATCGAGTTGATAGCTCGCGGT-BHQ-1 3’ were designed. The real time PCR cycling condition were 4 min preliminary heat at 95°C, 35 cycles of 30 s at 94°C, 30 s at 64°C and 30 s at 72°C with final extension for 5 min at 72°C (Bora et al., 2011). In duplex PCR, the capripoxvirus forward and reverse primers were 5’-AACTGCCGCTCAATGAAGAATGG-3’ and 5’TTTCAAAGCTTGTTTTATCGTGGG-3’ (413bp). The Orf virus forward and reverse primers were 5’AGGCGGTGGAATGGAAAGA3’ and 5’CCAGCAGGTATGCCAGGATG-3’ (708bp). The duplex PCR cycling condition were preliminary heat for 4 min at 94°C, 10 cycles of 30s at 94°C, 45s at 55°C and 1 min at 72°C, 20 cycles of 30 s at 94°C, 45 s at 57°C and 1 min at 72°C and final 10 min at 72°C (Zheng et al., 2007). DNA sequences of the obtained PCR products were analyzed by comparing with those in GenBank (Ali et al., 2013). Furthermore, differential diagnosis of foot and mouth disease (FMD) having similar symptoms to orf virus was determined by real-time RT PCR.

Results: All 4 scab samples detected by real-time PCR (Figure 2) and duplex PCR were positive for orf virus (Figure 3). Additionally, the DNA sequences of PCR products were consistent to sequences of orf virus genome (Figure 4). On further, differential diagnosis were negative for capripox virus and FMDV.
Conclusions: The young goat is susceptible to orf virus. The morbidity of the disease may reach 100% and, due to secondary infection, mortality may reach 15% (Ramesh et al., 2008). Most infected animals develop protective immunity, whereas reinfection is possible. Orf virus is highly zoonotic and may produce lesions. Treatment of individually affected animals is not provided unless lesions are severe and always consult a veterinarian (Lincoln University, no date). This study showed that the real-time PCR is a specific method for orf virus detection.

Keywords: orf virus, contagious ecthyma, real time PCR, duplex PCR, goat

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Figure 4  Phylogenetic analysis based on P55 gene
Multilocus Sequence Typing Analysis of Melissococcus plutonius Isolated from European and Japanese Honeybees: Spread of some Sequence Types Across Borders and Bee Species

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Introduction: Melissococcus plutonius is an important bacterial pathogen of honeybee larvae and causes European foulbrood (EFB) not only in European honeybees (Apis mellifera) but also in other native honeybees, such as Apis cerana and Apis laboriosa. We recently confirmed the first EFB case in Japanese native honeybees (Apis cerana japonica) and isolated M. plutonius from this case. In this study, to obtain a better understanding of the ecology of M. plutonius and the epidemiology of EFB, we analyzed M. plutonius isolates that originated from European and Japanese honeybees in Japan using a multilocus sequence typing (MLST) scheme and investigated the relationships of M. plutonius isolates from different honeybee species and different countries.

Methods: Isolated M. plutonius was identified as typical or atypical isolates by the duplex M. plutonius-specific PCR (Arai et al., 2014). MLST was performed by sequencing four genes as described previously (Haynes et al., 2013). The allelic numbers and sequence types (STs) of the isolates were determined by comparing their sequences with those in the M. plutonius MLST database (http://pubmlst.org/mp plutonius/). Novel alleles and STs were assigned through submission of the data to the database. Potential patterns of evolutionary descent between STs were calculated using the goeBURST algorithm (Francisco et al., 2009) in the PHYLOViZ program (Francisco et al., 2012). When constructing the goeBURST tree, MLST data of 86 isolates from this study were added to those of 61 isolates from Haynes et al. (2013) and 205 isolates from Budge et al. (2014).

Results: The analyzed Japanese isolates were resolved into six STs (ST3, ST4, ST12, ST25, ST26, and ST27), three of which (ST25, ST26, and ST27) were novel STs. Among the six STs, ST3 and ST12 were the two most common in Japan and found in isolates from both European and Japanese honeybees (or their environment). Of 27 STs identified so far, seven were found to contain isolates from more than one country. Among them, ST3 was the most globally distributed type and found in eight countries including Japan. ST4 found in a Japanese honeybee larva in Japan was identified in France and Australia. In addition, ST12 found in both Japanese and European honeybee larvae in Japan was found in the USA and the UK. Moreover, although ST26 has been identified only in Japan, this ST was a single-locus variant of ST13, and ST13 was found in Denmark, Poland, and the UK (Haynes et al., 2013; Budge et al., 2014). These results suggest the spread of some STs across borders and different Apis species.

Conclusions: We analyzed M. plutonius isolates isolated from European and Japanese honeybees in Japan by MLST and showed the spread of some STs across borders and honeybee species.

Acknowledgements: All data presented in this study have been reported in our previous publication (Takamatsu et al., 2014).

Keywords: European foulbrood, Japanese honeybee, European honeybee, Melissococcus plutonius, MLST

References


Characterization of Virulence-Resistance Plasmids Carried by Emerging Multidrug Resistant Salmonella Typhimurium Isolated from Cattle in Hokkaido, Japan

Virulence-resistance plasmids in S. Typhimurium

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Introduction: *Salmonella enterica* serovar Typhimurium is a common cause of salmonellosis in humans and animals. In 2002, we identified a multidrug-resistant clone with a pulsed-field gel electrophoresis (PFGE) pattern designated PFGE cluster VII disseminating among cattle in Hokkaido. These isolates carry the *bla*TEM-1 gene encoding TEM-1 β-lactamase on *S. Typhimurium* serovar-specific virulence plasmids. One isolate is resistant to the third-generation cephalosporin, cefotaxime, and harbors a *bla*CMY-2-carrying plasmid besides a virulence-resistance plasmid. In this study, we determined complete sequences of novel virulence-resistance plasmids and a *bla*CMY-2-carrying plasmid from *S. Typhimurium* isolates classified as PFGE cluster VII.

Methods: Plasmid DNA was sequenced by Hokkaido System Science Ltd. (Sapporo, Hokkaido, Japan) using the RocheFLX System (Roche Applied Science, Mannheim, Germany). Plasmid sequences were annotated using DDBJ Microbial Genome Annotation Pipeline ver. 1.06 (https://migap.lifesciencedb.jp/mgap/jsp/index.jsp). Sequence alignments were generated using BLAST, and plasmid sequences were compared using the Artemis Comparison Tool (ACT) (http://www.sanger.ac.uk/resources/software/act/).

Result and Discussion: Virulence-resistance plasmids pYT1 and pYT2 are 112,670-base pairs (bp) and 132,842-bp long, respectively, and closely related to pSLT, a virulence-associated plasmid essential for systematic invasiveness of *S. Typhimurium* in mice. The pYT1 and pYT2 sequences consist of segments derived from pSLT DNA and single large inserts (32,495 bp in pYT1 and 52,666 bp in pYT2) bracketed by two copies of IS1294. The inserted DNA in pYT2 has left and right region. The pYT1 sequence is identical to that of pYT2, except that it lacks the left region including plasmid-maintenance genes and the IncFIB replicon. The right region common to pYT1 and pYT2 harbors Tn21-like transposon containing a class 1 integron, which carries the resistance genes *aadA1, qacEΔ1*, and *sul1* (resistance to streptomycin-spectinomycin, ammonium antiseptics, and sulfonamides, respectively). The Tn2 transposon carrying *bla*TEM-1 (ampicillin resistance) is located upstream of the right-terminal IS1294. The *aph (3')-I* (kanamycin resistance) is located upstream of the left-terminal IS1294 in the right region. The *tetA* (tetracycline resistance) is located between *aph(3')-I* and the Tn21-like transposon. A *bla*CMY-2-carrying plasmid designated pYT3 is 121,723 bp, including *bla*CMY-2 and five antimicrobial-resistance genes as follows: *floR* (chloramphenicol/florfenicol resistance), *tetA* (A), *strA, strB* (streptomycin resistance), *sul2* (sulfonamide resistance). A 24,207-bp sequence in pYT3 contains the IncFIB replicon, which is flanked by two copies of IS1294. This segment is similar to the left region in pYT2. Although pYT3 shares sequence identity with the IncA/C plasmid pAR060302, no replication gene other than IncFIB was identified. Therefore, pYT3 may have been generated by IS1294-mediated recombination between segments in pAR060302 and the IncFIB replication region in the pYT2-like plasmid. The IncA/C replication region might have been replaced by the IncFIB replication region in pYT3.

Conclusions: Virulence-resistance plasmids from isolates of *S. Typhimurium* PFGE cluster VII were derived from a virulence plasmid through acquisition of a large insert containing a resistance island flanked by IS1294 elements. A *bla*CMY-2-carrying plasmid from a cefotaxime-resistant isolate comprised a segment of pAR060302 and the replication region of a virulence-resistance plasmid. These results provide insights into the evolution of drug resistance in emerging clones of *S. Typhimurium*. 
Introduction: In response to a call for action from the Thai Ministry of Health in light of several human cases due to Q fever endocarditis (Pachirat et al., 2012) the Thai Ministry of Agriculture and Cooperatives (Department of Livestock Development, DLD - National Institute of Animal Health, NIAH) has begun a national surveillance program for Coxiella burnetii, the causative agent of Q fever, in ruminants, the most probable reservoir for human exposure. Because animals often fail to seroconvert despite chronic infection and because organism is shed in high numbers in grossly normal placenta, we chose to use grossly normal placenta as the sample and real-time polymerase chain reaction (PCR) as the diagnostic method.

Methods: Field site area: the focus of the study is the north-east region because Q fever endocarditis human cases were identified, there is high density of ruminant populations and consumption of ruminant placenta for food is practiced there. Sampling has been conducted in the north, west, and central regions because of the high density of ruminant farms there. Now, sampling is being expanded to cover all of Thailand. Specimen collection: the provincial and district veterinary officers visit farms, fresh markets, middleman merchants, slaughter-houses, and restaurants. Placentas are placed for taking pictures and gross examination to determine lesions. Questionnaires record: epidemiological data is collected with each placenta sample. Laboratory processing: the freshly chilled placentas are shipped to laboratories of NIAH, Bangkok, Thailand. They are equipped with class II biosafety cabinets and potentially infectious material waste handling facilities. DNA extraction processing: Cotyledons are minced into small pieces prior to process for nucleic acid extraction using the Qiagen DNA/tissue kit. Real Time PCR was procedured by the published protocol (Christensen et al., 2006). Data analysis: Data are merged with spatial data and analyzed by using ArcGIS 10.

Results: The novel concept proved successful in an initial pilot study and is being expanded to develop a risk map. The results of real time PCR showed approximately 81% positivity in buffalo (12/15), 75% in beef cows (235/312), 69% in dairy cows (207/299), 50% in sheep (2/4), 46% in meat goats (35/76), up to 100% in Dairy goat (5/5), and as little as to 20% in other ungulates (1 nyala/5*) *1 nyala, 1 goral, 1 barbary sheep, 1 donkey, and 1 bactrian camel in Chiang Mai.
Conclusions: Positive placentas indicate the possibility of Q fever infection in ruminants despite lacking of clinical signs, as it is well known that *Coxiella* can be shed during healthy normal deliveries. However, it must be noted that the process of taking the placenta is fraught with potential for contamination by the bedding, manure, and environment, so the positive result might be an over-estimation. Further studies should focus on strain comparison between *Coxiella burnetii* in ruminant and humans. Immunohistochemistry could definitively identify infected placentas.

**Keywords:** Q fever, ruminant, Thailand

**References**


A crucial event in the initiation of many bacterial infections is the adherence of the bacteria to host cells, and bacterial surface structures and their interactions with host cell receptors play an important role in this process. *Erysipelothrix rhusiopathiae* is the causative agent of swine erysipelas, which may cause acute septicemia or chronic endocarditis and polyarthritis. To study the pathogenic mechanism of the widespread vascular disease observed in the acute form of swine erysipelas, we investigated the role of phosphorylcholine (PCho), a component of the *E. rhusiopathiae* capsule, in bacterial adherence to porcine endothelial cells (PECs) *in vitro*. We found that adherence of *E. rhusiopathiae* strain Fujisawa to PECs was twice that of adherence to control COS-7 cells and that the adherence rates of PCho-defective mutants were approximately 30-50% lower than those of the Fujisawa strain. The adherence of the Fujisawa strain to COS-7 cells transfected with the porcine platelet-activating factor receptor (PAFR) gene, which encodes a G protein-coupled receptor that has been shown to directly bind to *Streptococcus pneumoniae* via PCho in the bacterial cell wall, was not enhanced. Treatment with a PAFR antagonist (WEB-2086) did not inhibit bacterial adherence to PECs. Incubation of the bacterial cells with an antibody against PCho or SpaA, a choline-binding protein anchored to PCho of the Fujisawa strain, reduced the adherence of the strain to PECs. This effect was not observed when PCho-defective mutants were used. These results suggest that *E. rhusiopathiae* adheres to PECs via PCho and SpaA and that the PCho-mediated adherence is independent of PAFR.
Development of a Novel PCR-based Method for Typing of Capsular Polysaccharide Synthesis Gene Clusters of *Streptococcus suis*

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**Introduction:** *Streptococcus suis* is an important zoonotic bacterium leading to significant worldwide economic losses in the swine industry and causing a serious public health issue particularly in Asia. *S. suis* strains have been classified into 35 serotypes on the basis of antigenic differences in their capsular polysaccharide (CPS). Serotyping of *S. suis* is one of the most useful methods for the epidemiology of a particular outbreak and the monitoring of the prevalence of potentially hazardous strains. However, serotyping with all 35 typing antisera is time-consuming, and preparing the antisera is not easy due to the high cost and labor associated with its production. Therefore, the development of more practical and easier serotyping methods is desired.

**Methods:** First, we sequenced and analyzed the capsular polysaccharide synthesis (*cps*) gene clusters of all 35 serotype reference strains, and identified *cps* genes conserved among several serotypes and those specific to respective serotypes by clustering analysis with the gene family method implemented in PGAP-1.01 (Zhao et al., 2012). Then, PCR-based genotyping method, named as *cps* typing, was designed, and its usefulness was validated using 483 serotyped isolates from diseased and healthy animals and human patients.

**Results:** The developed *cps* typing method could determine *cps* types using only two multiplex PCRs. The first PCR (Grouping PCR) detected *cps* genes conserved in multiple serotypes and classified the tested strains into seven groups. The second PCR (Typing PCR) detected *cps* genes specific to the respective serotypes of each group and identified the *cps* type of the isolate. Our *cps* typing accurately assigned all serotype reference strains and nearly all of field isolates to the *cps* types predicted from their serotypes, although serotypes 1 and 1/2 could not be distinguished from serotypes 14 and 2, respectively, owing to the possession of the almost identical *cps* gene clusters. Furthermore, our method could type several non-typeable isolates by serotyping, many of which were considered to be acapsular due to the mutation(s) in the *cps* gene(s).

**Conclusions:** We developed a practical and easy two-step multiplex PCR assay to help serotyping of *S. suis*. The assay accurately typed almost all of the serotype reference strains and field isolates of various serotypes, and could also identify the genotypes of *cps* gene clusters of some serologically non-typeable strains.

**Keywords:** *Streptococcus suis*, Genotyping, *cps* gene cluster, Serotyping

**Reference**
Seroprevalence and Risk Factors of *Toxoplasma gondii* infection in Goats and Sheep in the Western Thailand

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Introduction: *Toxoplasma gondii* is a zoonotic, obligate intracellular protozoan parasite that causes toxoplasmosis and is capable of infecting a variety of animals and humans. Goats and sheep are susceptible to infection than the other animals. Toxoplasmosis also causes public health problems from consumption of infected meat and milk. This study aimed to determine the seroprevalence of *T. gondii* infection in goats and sheep in western provinces of Thailand and to identify potential risk factors associated with farms and goats and sheep seropositivity.

Methods: A cross-sectional study to determine the seroprevalence of *T. gondii* infection was conducted in samples derived from goats and sheep of 5 provinces in western Thailand namely; Kanchanaburi, Nakhonpathom, Prachuapkhirikhan, Petchaburi and Ratchaburi between January and July 2012. A total of 1,770 serum samples from 116 farms divide by 1,379 samples from 87 goat farms and 391 samples from 29 sheep farms were collected by stratified random sampling method. The serum samples were examined for *T. gondii* specific antibodies using indirect ELISA. Studies of risk factors for *T. gondii* infection in goats and sheep on individual and herd levels by using data from a questionnaire were performed. Risk factors including gender, age, herd size, free raising grass, mixed rearing goats and sheep, water from the public supply, cat and dog on farm, reproductive problem, neurologic problem and abortion problem, was analyzed by univariate analysis, and variables significant at *p* value < 0.05 were included in multivariate logistic regression models.

Results: The overall seroprevalence of *T. gondii* infection in goats on individual and herd levels were 17.04% and 83.91%, respectively and seroprevalence in sheep on individual and herd levels were 13.55% and 72.41%, respectively. The highest on individual and herd levels prevalence was observed in Kanchanaburi province. (Table 1) Logistic regression analysis in goats showed that neurologic problems observed (OR=1.682; 95%CI=1.026-2.760; *p*=0.039) and a large herd size (OR=1.391; 95%CI=1.046-1.850; *p*=0.024) were risk factors associated with *T. gondii* positivity on individual level. (Table 2) In sheep no risk factors was found to be associated with *T. gondii* positivity on individual level. However, no risk factors were associated with the presence of antibodies against *T. gondii* in goats and sheep on herd level.

Conclusions: The result indicated that *T. gondii* infection is found relatively high in goats and sheep rearing in western provinces of Thailand. A large flock size and neurologic problems observed are potential risk factors for *T. gondii* infection in goats. Therefore, surveillance and control measures should implemented by screening test then confirm with a molecular technique and culling of positive animals with should be established in order to reduce the risk of *T. gondii* infection in animals. Control and prophylactic measures must be adopted to improve the rearing system and the implementation of health promoting programmes in a joint effort between farmers and veterinarians.

Table 1 Seroprevalence of *Toxoplasma gondii* infection in goats and sheep in 5 provinces of western part of Thailand in January-July 2012

<table>
<thead>
<tr>
<th>Province</th>
<th>Prevalence</th>
<th>Goats</th>
<th>Herd</th>
<th>Goats</th>
<th>Herd</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Individual (n=1,379)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kanchanaburi</td>
<td>20.33(73/359)</td>
<td>87.50(21/24)</td>
<td>13.00(13/100)</td>
<td>83.33(5/6)</td>
<td>18.74(86/459)</td>
</tr>
<tr>
<td>Nakhonpathom</td>
<td>13.04(21/161)</td>
<td>81.82(9/11)</td>
<td>13.33(12/90)</td>
<td>83.33(5/6)</td>
<td>13.15(33/251)</td>
</tr>
<tr>
<td>Petchaburi</td>
<td>14.22(30/211)</td>
<td>100.00(11/11)</td>
<td>16.46(13/79)</td>
<td>50.00(3/6)</td>
<td>14.83(43/290)</td>
</tr>
<tr>
<td>Prachuapkhirikhan</td>
<td>16.31(77/472)</td>
<td>76.67(23/30)</td>
<td>15.56(7/45)</td>
<td>80.00(4/5)</td>
<td>16.25(84/517)</td>
</tr>
<tr>
<td>Ratchaburi</td>
<td>19.32(34/176)</td>
<td>81.82(9/11)</td>
<td>10.39(8/77)</td>
<td>66.67(4/6)</td>
<td>16.60(42/253)</td>
</tr>
<tr>
<td>Total</td>
<td>17.04(235/1,379)</td>
<td>83.91(73/87)</td>
<td>13.55(53/391)</td>
<td>72.41(21/29)</td>
<td>16.27(288/1,770)</td>
</tr>
</tbody>
</table>
Table 2 Final logistic regression model for positive risk factors associated with *Toxoplasma gondii* infection in goats on animal level

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>SE</th>
<th>P</th>
<th>Odds</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurologic problems observed</td>
<td>0.520</td>
<td>0.252</td>
<td>0.039</td>
<td>1.682</td>
<td>1.026-2.760</td>
</tr>
<tr>
<td>Herd size</td>
<td>0.330</td>
<td>0.146</td>
<td>0.024</td>
<td>1.391</td>
<td>1.045-1.850</td>
</tr>
</tbody>
</table>

B: regression coefficient, SE: standard error, CI: confidence interval at 95%

**Keywords:** seroprevalance, risk factors, *Toxoplasma gondii*, goats and sheep, western Thailand

**References**


External Parasites of Cultured Freshwater Fish in Central Region of Thailand during 2011-2013

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Introduction: External parasitic infestation is a common problem reported in tropical freshwater fish. It may cause weakness, growth retardation, as well as secondary infected wounds. However, a small numbers of parasite are frequently found in gill and skin of healthy fish. The harm caused by parasites may vary depending on inducing factors such as poor water quality, fish pond management problems which include high temperature phenomena that accelerate parasite growth and reproduction. Objective of this study to address the situation of external parasitic infestation in fish in Thailand.

Methods: We conducted a retrospective study in fish samples submitted to the National Institute of Animal Health from 5 provinces in central region of Thailand including Bangkok, Suphan Buri, Pathum Thani, Lop Buri and Chachoengsao during 2011 to 2013. The study was performed in 534 freshwater fish. External parasites were examined and identified by gill biopsy and skin scraping techniques under light microscope.

Result: The results revealed that 37.45% of the freshwater fish samples were positive for external parasitic infestation. The parasites found in this study could be divided into 4 groups and defined in 8 genera including Trichodina spp. (19.29%), metacercariae of digenea in family Heterophyidae (10.11%), Dactylogyrus spp. (8.43%), Epistylis spp. (2.81%), Gyrodactylus spp. (2.43%), Oodinium spp. (1.69%), Ichthyophthirius multifiliis (0.75%) and Glochidia (0.56%), shown in table 1.

Conclusions: The results suggested that external parasite is a common problem in fish in Thailand. The samples collected from Bangkok and Suphan Buri, mostly from fish hatchery farms, were found to be highly parasitic infestation (46.51% and 45.10%, respectively). A possible cause to young fish having inadequate immunity to protect themselves from external parasites could be affected by the high waste products resulted from high density stocking in cement ponds of the fish hatchery farms. Additionally, the stressful condition could lead to external parasitic co-infection in one fish that found in the present study as well. An unsurprising result obtained from the study is that external parasites infested in freshwater fish are more likely to be found in gills compared to skins (35.02% and 7.68%, respectively), as gill is a major organ in oxygen exchange and microorganism trap functions. Concerning to this point, external parasite should be carefully observed in gill. But some parasites, e.g. Epistylis spp., are frequently found in skin scraping, therefore laboratory diagnosis of the parasite is able to be performed accurately by examination in both gill and skin. The outcome of this study is expected to provide information of external parasitic data in freshwater fish that leads to the planning of treatment, control and prevention strategies in freshwater farms.

Keywords: external parasite, freshwater fish, central region of Thailand

Table 1 External parasitic genera detected in gill and skin samples from 534 fish

<table>
<thead>
<tr>
<th>External parasite genera</th>
<th>No. of fish infected</th>
<th>Sample detected in</th>
<th>Gill (%)</th>
<th>Skin (%)</th>
<th>Gill and Skin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protozoa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Trichodina spp.</td>
<td>103 (19.29)</td>
<td>61 (11.42)</td>
<td>27 (5.06)</td>
<td>15 (2.81)</td>
<td></td>
</tr>
<tr>
<td>- Oodinium spp.</td>
<td>9 (1.69)</td>
<td>9 (1.69)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>- Ichthyophthirius multifiliis</td>
<td>4 (0.75)</td>
<td>4 (0.75)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>- Epistylis spp.</td>
<td>15 (2.81)</td>
<td>1 (0.19)</td>
<td>12 (2.25)</td>
<td>2 (0.37)</td>
<td></td>
</tr>
<tr>
<td>Monogenea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Dactylogyrus spp.</td>
<td>45 (8.43)</td>
<td>45 (8.43)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>- Gyrodactylus spp.</td>
<td>13 (2.43)</td>
<td>10 (1.87)</td>
<td>2 (0.37)</td>
<td>1 (0.19)</td>
<td></td>
</tr>
<tr>
<td>Digenea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Family Heterophyidae metacercariae</td>
<td>54 (10.11)</td>
<td>54 (10.11)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Mollusca</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Glochidia</td>
<td>3 (0.56)</td>
<td>3 (0.56)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>246</td>
<td>187/534 (35.02)</td>
<td>41/534 (7.68)</td>
<td>18/534 (3.37)</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1  *Trichodina* spp.

Figure 2  Encysted metacercariae in family Heterophyidae trematode

(From Prof. Dr. W. Koerting)

Figure 3  *Oodinium* spp.

Figure 4  *Dactylogyrus* spp.

Figure 5  *Gyrodactylus* spp.

Figure 6  *Epistylis* spp.

Figure 7  *Ichthyophthirius multifiliis*

Figure 8  Glochidia
Development of Portable near-infrared (NIR) Instruments and System for Rapid and Easy Measurement of Blood Packed Cell Volume (PCV) in cattle

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*Corresponding author

Introduction: Bovine theileriosis is a serious disease that affects grazing cattle in Japan. A major symptom of this disease is anemia, and early detection of anemia on the basis of PCV (or the so called hematocrit value) measurement is vital for both saving animal life and preventing pandemic spread in the herd. PCV is usually measured by microhematocrit method or automatic blood cell counter, however, it takes much time especially in the field condition. In this study, we attempted to develop a rapid and easy PCV measurement system in the field. We developed and used specially designed probes and NIR instruments for non-invasive and invasive measurement of PCV in cattle.

Materials and methods

i) non-invasive measurement

60 Japanese Short-horn cattle (beef) were used. NIR spectra and blood for PCV analyses were obtained. NIR spectra were acquired using an originally designed portable NIR instrument for non-invasive measurement of cattle [Fig. 1] based on a multichannel spectrometer (S-2930, SOMA Optics, Ltd., Tokyo, Japan). These spectra were acquired from the tail area [Fig. 1] approximately three times. The instrument was operated in the short near-infrared wavelength region from 700 nm to 1050 nm at 1 nm intervals. An optical fiber probe for tail measurement was developed to ensure a 20 mm distance between the halogen bulb illuminator and detector. Each NIR spectrum was taken within 10 seconds from cattle.

ii) invasive measurement

912 cattle (beef and dairy) blood samples were obtained at several pastures in Japan. The blood samples (about 2 mL) were drawn from jugular vein or tail vein/artery and kept them in the vacuum blood collection tubes contained anticoagulant EDTA (outer diameter of 13.2 mm, VP-NA052K, TERUMO, Co. Ltd., Tokyo, Japan). Short near-infrared wavelength (from 700 nm to 1050 nm at 1 nm intervals) spectra of the blood samples were measured by a portable NIR spectrometer just after the blood was drawn. The spectrometer was originally designed for field measurements based on a multichannel spectrometer (S-2930, SOMA Optics, Ltd., Tokyo, Japan) equipped with a touch panel and Li-ion battery [Fig. 2]. Standard vacuum blood collection tubes were considered applicable for sampling for the purposes of spectroscopy. In order to obtain sufficient light intensity, five halogen bulbs were arranged radially around the tube. Each NIR spectrum was taken within 10 seconds from cattle.

iii) Data analysis

Calibration model were developed by partial least-squares (PLS) regression using the Unscrambler (CAMO, Oslo Norway) or Matlab (The MathWorks inc., Natic, MA, USA). Before starting the PLS regression, raw spectra were pre-treated in order to remove baseline shift due to scattering and noise as well.

iv) Reference analysis

PCV were measured using two methods: by centrifugation (PCV(CT)) and using the automatic blood cell counter (Celltac α, MEK-6450; Nihon Kohden Co., Tokyo, Japan) (PCV(BC)).

Results and discussion

i) non-invasive measurement

The PCV(CT) and PCV(BC) given by two methods showed average about 33%, ranging from 22% to 48% [Table 1]. Low-noise spectra were obtained from each animal. Table 2 shows PLS calibration results for PCV(CT) and PCV(BC) using NIR spectra measured at the tail. The coefficient of determination (R2) and the root mean square errors of prediction (RMSEP) of PCV(CT) were determined to be 0.55 and 3.03%, respectively, and those of PCV(BC) were 0.57 and 3.09%, respectively [Table 2]. Further development on the probe used to acquire spectra at the blood vessel located on tail of animal should be carried out, especially to control the lamp intensity and distance between the illuminator and detector.

ii) invasive measurement

The PCV(CT) showed normal distribution for all 912 samples, ranged from 16.5% to 50% and average 31.8% [Table 1]. The spectra showed a distinctive absorption maximum between the blood sample taken from vein or artery. The coefficient of determination and the RMSEP of PCV(CT) were determined to be 0.82 and 1.69%, respectively [Table 2]. This result indicates that this measurement system will be a useful tool for field examination in pastures and has a possibility to replace the time-consuming conventional centrifuge method.
Table 1 Chemical characteristics of blood samples used for developing calibration equations.

<table>
<thead>
<tr>
<th>Items</th>
<th>N</th>
<th>Average</th>
<th>Standard Deviation</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV(CT)(%)</td>
<td>60</td>
<td>33.3</td>
<td>4.2</td>
<td>22.0</td>
<td>47.0</td>
</tr>
<tr>
<td>PCV(BC) (%)</td>
<td>60</td>
<td>33.9</td>
<td>4.2</td>
<td>22.9</td>
<td>48.2</td>
</tr>
</tbody>
</table>

ii) invasive measurement

<table>
<thead>
<tr>
<th>Item</th>
<th>N</th>
<th>Average</th>
<th>Standard Deviation</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV(CT)(%)</td>
<td>912</td>
<td>31.8</td>
<td>4.0</td>
<td>16.5</td>
<td>50.0</td>
</tr>
</tbody>
</table>

Table 2 PLS calibration results for predicting PCV.

i) non-invasive measurement

<table>
<thead>
<tr>
<th>Items</th>
<th>Unit</th>
<th>F</th>
<th>R²</th>
<th>SECV</th>
<th>RMSEP</th>
<th>Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV(CT)</td>
<td>%</td>
<td>5</td>
<td>0.55</td>
<td>2.71</td>
<td>3.03</td>
<td>0.00</td>
</tr>
<tr>
<td>PCV(BC)</td>
<td>%</td>
<td>6</td>
<td>0.57</td>
<td>2.63</td>
<td>3.09</td>
<td>-0.03</td>
</tr>
</tbody>
</table>

ii) invasive measurement

<table>
<thead>
<tr>
<th>Item</th>
<th>Unit</th>
<th>F</th>
<th>R²</th>
<th>SECV</th>
<th>RMSEP</th>
<th>Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV(CT)</td>
<td>%</td>
<td>5</td>
<td>0.83</td>
<td>1.60</td>
<td>1.69</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Conclusions:

This study aimed to develop a portable NIR instrument for non-invasive and invasive measurements and demonstrate its application in determination of PCV in cattle. A specially designed probe and NIR instruments have the potential to detect anemia cattle in pastures non-invasively. Invasive measurement system will be a useful tool for measuring PCV easily and quickly in the field.

This research has been supported by the Grain-in-Aid of Research and Development Projects for Application in Promoting New Policy of Agriculture, Forestry and Fisheries (#22020) funded by Ministry of Agriculture, Forestry and Fisheries of Japan.

Keywords: anemia, cattle, near-infrared(NIR), non-invasive measurement, PCV
Nitrate Poisoning due to Ingestion of *Mimosa invisa* in Cattle

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Introduction: *Mimosa invisa* is a shrubby herbaceous plant and a native plant to tropical America. Currently, *Mimosa invisa* widely distributes in Southeast Asia especially Thailand, which is commonly found along road sides and in moist waste areas (Alex et al., 1991). Moreover, it is a serious weed and a nitrogen-fixer plant, which can accumulate nitrate and cause severe toxicity in animals. Although nitrate in itself is not toxic to animals, it can become toxic resulting from nitrite. Nitrate poisoning is usually associated with animals ingesting forage or plants with a high nitrate content. Ruminants are susceptible to this poisoning because of their high level of nitrate-reducing microbes in the rumen (Clark et al., 1981; Rajan et al., 1986). The reduction of nitrate to nitrite occurs much more rapidly in rumen than does the nitrite to ammonia; consequently, nitrite is absorbed into red blood cells and converts hemoglobin to form methemoglobin, which is incapable of carrying oxygen to tissue and results in hypoxia (Stan and Tim, 2003). The aim of this study was to describe nitrate poisoning in cattle caused by ingestion *Mimosa invisa* which is considered a toxic plant.

Methods: A herd of cattle at different ages in Buriram province, Thailand were fed by fresh *Mimosa invisa*. Approximately 12 to 15 hours after plant consumption, cattle showed clinical signs including aggressive movement, foam in mouth, ataxia, ascites and weakness. Nine cattle were found dead in the following morning. Three out of nine cattle were performed necropsy and gross lesions were observed then internal organs such as heart, liver, and fresh *Mimosa invisa* were collected for toxicology analysis. Nitrate contamination was determined by spectrophotometer (Badiadka and Kenchaiah, 2009). The level of nitrate at 5,000-10,000 mg/kg (dry matter) or higher in forages are considered capable of producing toxicity in cattle whereas nitrate concentration exceeded 20 mg/kg found in specimens are generally considered diagnostic for excessive nitrate exposure (Stan and Tim, 2003). Simultaneously, pesticides and cyanide were examined.

Results: Gross lesion observation was associated with ruminal tympany, hemorrhages in heart and kidney, and congestion of the rumen and abomasum. The average amount of nitrate in *Mimosa invisa* was 6,054 mg/kg for dry matter and 1,327 mg/kg for wet matter whereas the average amount of nitrate in heart, liver and kidney were 44, 74 and 85 mg/kg, respectively which were high level. Nitrate in rumen content were range between 147-243 mg/kg in wet matter. Pesticides and cyanide were not found.

Conclusions: Diagnosis of nitrate poisoning was based on observation of clinical signs and the possibility of exposure to toxic plants. In this report, the concentration of nitrate found in suspected samples is consistent with the clinical signs of nitrate poisoning. Therefore, nitrate poisoning by the consumption of *Mimosa invisa* was the cause of death and this problem can be avoid with proper management of forage and livestock.

Keywords: Nitrate poisoning, *Mimosa invisa*, cattle

References
Changes in the Concentration of Fumonisins in Forage Rice during the Growing Period, Differences among Cultivars and Identification of the Causal Fungus

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1National Institute of Animal Health, 2Institute of Livestock and Grassland Science (NILGS), 3National Institute for Agro-Environmental Sciences, Tsukuba, Ibaraki 305-8604 Japan

*This experiment was done when the first author was enrolled in NILGS.
*Corresponding author

Introduction: Mycotoxin contamination of crops is a global problem for the health of the humans and livestock who consume these crops. In Japan, fumonisins (FUMs) are a class of mycotoxins that pose a serious concern because of their high frequency of detection in crops.

In Asia, rice (Oryza sativa L.) is one of the most popular crops and serves as a staple food. Furthermore, in recent years, forage rice has been developed for use as animal fodder. To clarify changes in the concentration of fumonisins in forage rice during the growing period, we conducted field experiments to determine changes in FUM contents during forage rice cultivation, differences among forage rice cultivars, and the identity of the main FUM-producing fungi.

Materials and methods: We used 10 cultivars; Hokuriku-193, Natsuaoba, Kusanohoshi, Fukuhibiki, Momiroman, Hidekomochi, Mochidawara, Takanari, Bekoaoba, Yumeaoba. Cultivation was done in a paddy field at the NILGS in Nasushiobara, Tochigi, Japan, in 2011 and 2012. The sampled biomass was separated into two parts: stems and leaves, and the heads. Sampling was done in triplicate.

Isolation of FUM-producing fungi was carried out in 2011. We extracted total genomic DNA from the FUM-producing fungus isolates, and amplified part of the gene for translation elongation factor 1-α (EF-1α) by means of the polymerase chain reaction (PCR). The sequence data were compared with data in the DNA Data Bank of Japan.

The fresh plant samples were dried at 65 °C for 48 h so that all concentrations could be expressed per unit dry weight. The dried samples were ground in a cutting mill. FUMs were extracted and purified, and injected into a liquid chromatography–tandem mass spectrometry (LC/MS/MS) system.

Results and discussion: The FUM concentrations in the stems and leaves were lower than head (Figure 1). In the heads, the FUM concentrations in all cultivars 30 days after heading were less than 10 µg/kg DM. However, by 40 days after heading, the FUM concentration began to increase. The FUM concentration reached its maximum around 40 to 70 days after heading. Fukuhibiki had a significantly higher FUM concentration than Kusanohoshi, Momiroman, Mochidawara, Takanari, Bekoaoba and Yumeaoba. Fukuhibiki and Natsuaoba had a significantly higher FUM concentration than Mochidawara. The other cultivars did not differ significantly. Although the maximum concentration differed among the cultivars, the trend did not differ. We thought that the increased FUM concentration in rice heads may be a response to changes in some nutritional component in these tissues.

We obtained 49 Fusarium isolates that were confirmed to produce FUM, and Fusarium fujikuroi was the main fumonisin producer. The isolation frequency was greatest at 51 days after heading. These fungi were also isolated from both parts include of the stems and leaves, although the FUM concentrations were quite low compared with those in the heads. These differences may have resulted from differences in the nutrient compositions of the parts of the host plants.

Keywords: Fumonisins, Mycotoxin, Forage rice, Fusarium fujikuroi, Livestock feed

![Figure 1](Changes in the fumonisin (FUM) concentration in the 10 cultivars during cultivation at the NILGS. FUM concentrations represent the total (FUMB_{1}+FUMB_{2}) content.)

Oral Presentation
Introduction: Currently, pineapple peel from pineapple can industry is widely used for dairy cattle feed, due to cheap and available all year round. However, the residue of herbicide and insecticide used during cultivation of pineapple may be harmful to dairy cattle. Therefore, we conducted a survey of pesticides contamination in samples by using Thin Layer Chromatography(TLC) and Gas Chromatography/Mass Spectrometry(GC/MS) with three-way splitter.

Methods:
Sample Collection - Forty four pineapple peel samples and one axillary crownlet sample were collected from 12 dairy cattle farms in 3 districts of Prachuap Kirikhun province, Muang, Kuiburi and Panburi between June and December 2011.
Sample preparation - All samples were extracted using liquid-liquid extraction. The sample preparation included: weighing of 20 g sample in 500 ml Erlenmeyer flask. Add 100 ml of acetone and 50 ml of distilled water, shake 250 rpm for 30 min. Vacuum filtrate with Buchner funnel and then extraction with 100 ml methylene chloride 2 times. The extraction solution was evaporated until 5 ml remains (crude extract). Clean-up by column chromatography using florisil for packing column and eluted with 100 ml hexane : acetone(95:5) and 100 ml hexane : acetone(75:25) respectively. Evaporation and re-dissolution with 1 ml of acetone for TLC and GC/MS analysis.

Results: The results showed that organophosphorus and carbamate were not detected in all samples but diuron, one of the herbicides, in 3,4-dichloroaniline (3,4-DCA) metabolite form amount 1.58 mg/kg was found from one of axillary crownlet sample. In addition 67% (30/45) of the tested samples were positive with eugenol in the concentration of 0.11-2.05 mg/kg(Table 2). Statistically significant in amount of eugenol was observed between June and December(p<0.05).
Table 1 Limit of Detection (LOD), Limit of Quantitation (LOQ), and recovery of Organophosphorous, Carbamate, Eugenol and Diuron

<table>
<thead>
<tr>
<th>Substances</th>
<th>LOD (mg/kg)</th>
<th>LOQ (mg/kg)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organophosphorous</td>
<td>0.41-0.47</td>
<td>1.37-1.66</td>
<td>93-120</td>
</tr>
<tr>
<td>Carbamate</td>
<td>0.38-0.61</td>
<td>1.25-1.66</td>
<td>85-120</td>
</tr>
<tr>
<td>Eugenol</td>
<td>0.03</td>
<td>0.11</td>
<td>85</td>
</tr>
<tr>
<td>Diuron</td>
<td>0.42</td>
<td>1.38</td>
<td>110</td>
</tr>
</tbody>
</table>

Table 2 The results of pesticides in pineapple peel samples and crownlet sample of 3 districts in Prachuap Kirikhun province

<table>
<thead>
<tr>
<th>Substances</th>
<th>Muang district (n=13)</th>
<th>Kuiburi district (n=16)</th>
<th>Panburi district (n=16)</th>
<th>Total (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>June</td>
<td>December</td>
<td>June</td>
<td>December</td>
</tr>
<tr>
<td>Organophosphorous</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carbamate</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Eugenol</td>
<td>0.37-0.49</td>
<td>0.11-0.16</td>
<td>0.81-0.94</td>
<td>0.16-0.75</td>
</tr>
<tr>
<td>Diuron (3,4-DCA)</td>
<td>1.58</td>
<td>(1/7)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

- = not detected  
** = number of detected sample/number of sample  
n = number of sample

Conclusions:

- Organophosphorus and carbamate were not detected in all samples.
- 3,4-dichloroaniline (3,4-DCA) which is metabolite form of diuron amount 1.58 mg/kg was found from one of axillary crownlet sample. So farmers should be aware when they use axillary crownlet for dairy cattle feed. LD$_{50}$ (oral rats) of 3,4-DCA is 60 mg/kg.
- Most of pineapple peel samples were found eugenol which is one kind of phenolic compound that is found in plants containing essential oils such as citronella, clove, ginger, basil, etc. Eugenol is an inhibitor of antibacterial and antifungal. Though pineapple peel can use as dairy cattle supplement. However it can cause liver failure and nerve cell damage when use in high dosage.
- Further investigation should be done relevant to eugenol in terms of toxic dosage and releasing in milk to prevent animal and human health.

Keywords: pesticides, organophosphorus, carbamate, pineapple peel, GC/MS
Molecular Epidemiological Study of *Burkholderia pseudomallei* Isolated from Goats and Soil in Farm by Pulsed Field Gel Electrophoresis

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**Introduction:** *Burkholderia pseudomallei* causes of melioidosis in humans and many animals especially in Southeast Asia and northern Australia. In animals, goat and pigs are mostly reported for *Burkholderia pseudomallei* infection. Since this bacteria is highly tolerance in the environment especially in soil then the contaminated soil is concerned as a major cause of infection. The surveillance from bacterial laboratory of Southern Veterinary Research and Development Center, Thailand, found the continuous infection in one goat farm since 2007 in Songkhla province. Therefore this study aimed to analysis *B. pseudomallei* to determine the relation between infected goat and contaminated soil to find the possible source of infection in this farm.

**Material and methods:** Molecular epidemiology study of *B. pseudomallei* in a goat farm in Songkhla province was carried out. Total 15 strains of *B. pseudomallei* comprised 11 and 4 isolated from infected goats from 2007 to 2013 and soil in 2013. Pulsed field gel electrophoresis (PFGE) was performed to analyze the chromosomal DNA by using enzyme Spe I and Bio1D++. 

**Result:** DNA patterns appeared in 9 different genotypes (A, B, C, D, E, F, G, H and I). as show in Fig.1. Pattern A (3 strains) and B (1 strain) were DNA pattern of *B. pseudomallei* isolated from soil and others were from infected animals. The most frequent patterns were E and G (3 strains each).

**Conclusion:** Because none of DNA pattern from goats and soil was matched, so the outbreak of melioidosis in this farm is likely due to contaminating the secretions of sick animals rather than soil-borne infection.

**Keywords:** *Burkholderia pseudomallei*, DNA pattern, pulsed field gel electrophoresis

![Fig 1 DNA patterns appeared in a different genotypes (A,B,C,D,E,F,G,H and I)](image-url)
**Case Report: Co-incidence of Jaagsiekte Sheep Retrovirus and *Mannheimia haemolytica* Infections in a Sheep Farm in Phitsanulok Province.**

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**Introduction:** Ovine pulmonary adenocarcinoma (OPA), also commonly known as Jaagsiekte, is a contagious lung cancer of sheep (Griffiths et. al., 2010) which can cause economic loss from low production because of high mortality rate when its clinical signs appear. It is caused by Jaagsiekte sheep retrovirus (JSRV). Death is often precipitated by a superimposed bacterial pneumonia, particularly due to *Mannheimia haemolytica* infection (OIE, 2008). This report describes the clinical features of the co-incidence of OPA and *Mannheimia haemolytica* infections in a sheep in Phitsanulok province and methods of diagnosis which will be useful for veterinarians and farmers in lower northern region of Thailand to aware existence of OPA for the prevention, control and eradicate planning program.

**Methods:** History taking was performed to find out clinical signs for differential diagnosis after that the necropsy was conducted for gross findings. Tissue samples from the lung, heart, liver, kidney and spleen were collected and fixed in 10% buffered formalin, routinely processed, and stained with hematoxylin and eosin (HE) for histopathologic examination. After necropsy, those tissues were sent for bacterial culture and virus identification.

**Result:** From history taking, an 18-month old ewe died in a sheep farm in Bang-rakum district, Phitsanulok province. There are 90 sheep raised by free grazing in this farm. The animal showed signs of chronic respiratory disease, depression, anorexia, weight loss and seizures before death. At necropsy, it revealed that respiratory tracts and trachea were filled with frothy exudate. Pleura was covered by fibrin sheet and adhered with thoracic wall. Lungs were enlarged and edematous. In addition, there were scattered small grayish-white nodules of 1-3 cm. in diameter in lung parenchyma as shown in Fig.1.

Histopathology section of lung revealed the epithelium of alveolar had formation of neoplastic cells composed of one layer of columnar or cuboidal epithelial cells arranged in 2 pattern: acinar and papillary which protruded in respiratory space which is specific characterization of OPA. Moreover, the neoplasms were surrounded by a large number of mononuclear cells whereas alveolar and pleural space were infiltrated by numerous polymorphonuclear cells which can be caused by bacterial infection as shown in Fig.2.

JSRV, the etiological agent of OPA was amplified from neoplastic lung tissues by PCR technique (De las Heras et. al., 2005). The result from bacteria laboratory found *Mannheimia haemolytica* in lung tissue by isolation and PCR technique.

**Conclusion:** Base on evidence from histopathological lesions, viral identification and bacterial isolation. It was confirmed that the sheep was infected by JSVR and complicated with *Mannheimia haemolytica* which are the cause of death. OPA is usually introduced into a flock by an infected sheep who may be subclinically infected (Thongkamkoon et. al., 2011). Because there is no effective diagnostic test to detect the disease in live animals and preventing entry is difficult. Therefore, Herd replacements should be bought only from flocks with no history of OPA (CFSPH, 2009).

**Keywords:** Ovine pulmonary adenocarcinoma, *Mannheimia haemolytica*, Jaagsiekte sheep retrovirus, Sheep, Phitsanulok province.

**References**


Fig 1  The cut section of lung display the dense, various size, numerous grayish-white tumor with an uneven cobbled texture (A). Fibinous pleuritis: pleura was covered by fibrin sheet and adhered with thoracic wall (B).

Fig 2  The tumor is mixed with acinar and papillary pattern which protruded in respiratory space HE.40X (A). A large number of polymorphonuclear cell infiltration in alveolar space. HE. 20X (B).
Comparison of Serological Tests for Antibody Detection Against *Brucella abortus* Infection in Cattle and Buffaloes

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**Introduction:** *Brucella abortus* causes an important infectious disease and its occurrence has become worldwide. The control measures have largely relied on identification followed by eradication of infected herds. Rapid diagnosis is therefore crucial in allowing these control measures to be effective. Conventional methods for brucellosis diagnosis have long been used; however, they have their limitation in that a combined result from at least 2 methods is required for making a definitive diagnosis (Garin-Bastuji et al., 2006; OIE, 2008). This present study is aimed at comparing the sensitivity, specificity as well as other characteristics of four serological tests, including Rose Bengal test (RBT), EDTA-Serum Agglutination test (EDTA-SAT), Complement Fixation test (CFT) and indirect Enzyme Immunosorbent Assay of National Institute of Animal Health (I-ELISA-NIAH) for antibody detecting against *B. abortus* in cattle and buffaloes.

**Methods**

**Serum Samples:** Serum control 5,642 samples were used for diagnostic mean (Negative samples included 5,300 cattle sera from farms which neither history nor serological evidence of *B. abortus* infection were found and Positive samples included 342 cattle sera of which *B. abortus* infection had been confirmed by isolation). Field samples were 15,003 cattle sera and 3,804 buffalo sera collected from the central part of Thailand during 2012-2013.

**Antigens:** RBT and EDTA-SAT antigens were prepared from *B. abortus* (Bureau of Veterinary Biologics; BVB, Department of Livestock Development, Thailand). CFT antigen was prepared from *B. abortus* strain 99 (Antifix®, Synbiotics Corp. Lyon, France) and standardized against OIE International Standard Serum (OIEISS). I-ELISA-NIAH antigen was smooth lipopolysaccharide (sLPS) prepared from *B. abortus* strain 99 (Japan International Cooperation Agency) as described by Nielsen et al., 1996 and OIE (2008).

**Serological tests:** RBT was performed according to OIE (2008). EDTA-SAT was performed according to NIAH (2007). CFT was performed according to French Food Safety Agency (2009) and OIE (2008). I-ELISA-NIAH was performed according to Ekgatat et al. (2008; 2009).

**Test evaluation:** Diagnostic sensitivity (DSe), diagnostic specificity (DSp), positive predictive value (PPV), negative predictive value (NPV), accuracy (Ac) and kappa value (K) were calculated with respect to the *Brucella*-infected and uninfected groups (confirmed by isolation). Relative Se, Sp, Ac and K of RBT, EDTA-SAT and I-ELISA-NIAH (with respect to CFT as standard method) were tested on field cattle and buffalo sera. All values were calculated by using WinEpiScope, Version 2.0. For the degree of agreement of Kappa statistic the criteria is <0 (no agreement) and 0.81-1.00 (almost perfect agreement).

**Results**

**Diagnostic serological results:**

The results of the four tests using serum control (N = 5,642) were shown in Table 1.

<table>
<thead>
<tr>
<th>Method</th>
<th>DSe</th>
<th>DSp</th>
<th>PPV</th>
<th>NPV</th>
<th>Ac</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBT</td>
<td>99.71 (99.135-100.0)</td>
<td>99.76 (99.622-99.888)</td>
<td>96.33 (94.368-98.287)</td>
<td>99.98 (99.944-100.0)</td>
<td>99.75</td>
<td>0.979</td>
</tr>
<tr>
<td>EDTA-SAT</td>
<td>90.94 (87.893-93.979)</td>
<td>99.98 (99.944-100)</td>
<td>99.97 (99.052-100.0)</td>
<td>99.42 (99.214-99.623)</td>
<td>99.43</td>
<td>0.948</td>
</tr>
<tr>
<td>CFT</td>
<td>96.49 (94.541-98.441)</td>
<td>99.60 (99.435-99.773)</td>
<td>94.02 (91.536-96.498)</td>
<td>99.77 (99.645-99.901)</td>
<td>99.42</td>
<td>0.949</td>
</tr>
<tr>
<td>I-ELISA-NIAH</td>
<td>99.42 (98.607-100)</td>
<td>99.89 (99.796-99.977)</td>
<td>98.27 (96.890-99.641)</td>
<td>99.96 (99.910-100.0)</td>
<td>99.86</td>
<td>0.988</td>
</tr>
</tbody>
</table>
Relative serological results:
The results of the three tests with respect to CFT as standard method and using field cattle sera (N = 15,003) were shown in Table 2.

<table>
<thead>
<tr>
<th>Method</th>
<th>Se</th>
<th>Sp</th>
<th>PPV</th>
<th>NPV</th>
<th>Ac</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBT</td>
<td>92.95</td>
<td>99.60</td>
<td>85.32</td>
<td>99.82</td>
<td>99.43</td>
<td>0.887</td>
</tr>
<tr>
<td>EDTA-SAT</td>
<td>85.64</td>
<td>99.88</td>
<td>94.90</td>
<td>99.64</td>
<td>99.53</td>
<td>0.898</td>
</tr>
<tr>
<td>I-ELISA-NIAH</td>
<td>98.37</td>
<td>99.28</td>
<td>77.56</td>
<td>99.96</td>
<td>99.26</td>
<td>0.864</td>
</tr>
</tbody>
</table>

The results of the three tests with respect to CFT as standard method and using field buffalo sera (N = 3,804) were shown in Table 3.

<table>
<thead>
<tr>
<th>Method</th>
<th>Se</th>
<th>Sp</th>
<th>PPV</th>
<th>NPV</th>
<th>Ac</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBT</td>
<td>85.48</td>
<td>99.71</td>
<td>88.33</td>
<td>99.76</td>
<td>99.58</td>
<td>0.867</td>
</tr>
<tr>
<td>EDTA-SAT</td>
<td>66.13</td>
<td>99.97</td>
<td>97.62</td>
<td>99.44</td>
<td>99.42</td>
<td>0.786</td>
</tr>
<tr>
<td></td>
<td>(54.348-77.910)</td>
<td>(99.921-100.0)</td>
<td>(93.008-100.0)</td>
<td>(99.204-99.680)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-ELISA-NIAH</td>
<td>95.16</td>
<td>99.79</td>
<td>88.06</td>
<td>99.92</td>
<td>99.71</td>
<td>0.913</td>
</tr>
<tr>
<td></td>
<td>(89.820-100)</td>
<td>(99.638-99.934)</td>
<td>(80.295-95.824)</td>
<td>(99.829-100.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion:
There is no single serological test for diagnosis of brucellosis that can detect in all stages of disease. The diagnosis of brucellosis can be made by isolation and identification of *Brucella* and in the case of the bacterial culture is not practical, the diagnosis should be based on serological tests. The results of serological tests rely on the selected sera and the stage of the disease (OIE 2012). In this work, the results were as the preliminary analysis the performance of the serological tests and as the guide for the decision.

In this study, the result of RBT was shown DSe 99.71% and DSp 99.76% in cattle, this was similar and in the range of the previous studied in cattle, buffalo and human (Stemshorn *et al.*, 1985; Gomez *et al.*, 2008; Akhtar *et al.*, 2010). In cattle, EDTA-SAT was shown DSe 90.94% and DSp 99.98% whereas the previous studied as 68.9% and 99.2%, respectively (Stemshorn *et al.*, 1985). For CFT; the DSe was 96.49 % and DSp 99.60% which higher than Gall *et al.* (2000) who studied in bison (DSe 89.5% and DSp 95.5%). Whereas the I-ELISA in this study is nearly same in both DSe and DSp (DSe 100%, DSp 96.6%). So, for diagnosis of brucellosis, it can be used both RBT and I-ELISA as a screening test.

According to OIE Terrestrial Manual 2012, the CFT is one of the test that used as a prescribed test for brucellosis for international trade. In this study, we considered and estimated the relative Se and Sp in the field sera of cattle and buffaloes without gold standard and we used CFT as the abbot. The relative Se of the tests gave the following ranking: I-ELISA-NIAH>RBT>EDTA-SAT, whereas the rank of relative Sp was EDTA-SAT>RBT>I-ELISA-NIAH. It was revealed that the Se of RBT in cattle was in the same range of Muma *et al.* (2007) (93%) while in buffalo was lower than in cattle same as Montagnaro *et al.* (2008) (84.5%). For SAT in cattle (85.64%) and buffalo (66.13%), which was closed to Fosgate *et al.* (2002) (Cattle 80.2% and buffalo 75.0%). From this study, the EDTA-SAT was not suitable for screening and diagnosis in buffalo because of low Se even though the Sp quite high. In all test methods, it showed the Kappa value in good level of agreement (K value 0.81-1.00 = almost perfect agreement). Therefore, to desire what method to use, the method properties need to be identified.

Keywords: *Brucella abortus*, brucellosis, serological tests, cattle, buffaloes
A Serological Survey of Classical Swine Fever in the Eastern Region of Thailand

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Introduction: Classical swine fever (CSF) also known as hog cholera, is a contagious viral disease of domestic and wild swine. An outbreak of CSF has serious consequences for swine industry. Detection of antibodies is the method of choice for diagnosis of CSF in live pigs whereas detection of virus is most suitable when the pig is dead. In Thailand, CSF is one of the major causes of economic loss of the pig farms. CSF is a notifiable disease according to Thailand’s Animal Epidemics Act 1956 and its revision 2004 for animal disease control and eradication. Prevention and control of CSF are based on vaccination. Currently, three strains of modified live CSF vaccines as Chinese, GPE (-) and thiverval are used widespread which Chinese strain vaccine is the most widely used for the control of CSF. This study was to evaluate a quantitative CSF antibody in pig sera in the eastern region of Thailand using indirect ELISA

Methods: A total of 1,796 pig sera from 86 herds of 8 provinces in eastern Thailand between 2010 – 2013. were tested using commercial enzyme linked immunosorbent assay (ELISA). The commercial indirect ELISA kit has adequate sensitivity according to OIE criteria for CSF ELISA antibody tests. The indirect ELISA was used in measure the amount of antibody to the envelope protein E2 of CSF virus in pig sera at a dilution of 1:30 according to the manufacturer’s instruction (BioChek, UK) and read absorbance at 405 nm. Calculate the ratio of the mean absorbance of the test sample and mean of positive control (S/P). S/P ratios were recalculated in the end point antibody titer from each individual pig using the formula: Log₁₀ titer = 1.1 x Log₁₀ S/P + 3.361 where antibody titer = antilog of Log₁₀ titer. Antibody titer greater or equal to 10⁷.¹ is considered positive. The results were expressed as positive or negative (Table 1).

Table 1 Interpretation of results

<table>
<thead>
<tr>
<th>S/P value</th>
<th>Titer Range</th>
<th>Antibody status</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.499 or less</td>
<td>1070 or less</td>
<td>Negative</td>
</tr>
<tr>
<td>0.500 or greater</td>
<td>1071 or greater</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Calculation of S/P ratio = mean of test sample - mean of negative control
mean of positive control - mean of negative control

Calculation of antibody titer

\[ \log_{10} \text{titer} = 1.1 \times \log_{10} S/P + 3.361 \]

\[ \text{Antilog} = \text{titer} \]

Results: CSF antibody in pig sera were detected in 84 of 86 herds (97.7%). Out of 1,796 sera examined, 1,586 (88.3%) indicated positive results with high antibody titer. Overall mean antibody titer from 2010-2013 as assessed by CSFV E2 ELISA kit were 6230, 5658, 3921 and 4723, respectively (Table 2).

Table 2 The presence of CSF antibody in pig sera by CSFV E2 ELISA in the eastern region of Thailand during 2010-2013.

<table>
<thead>
<tr>
<th>Year</th>
<th>% (positive/herd)</th>
<th>% (positive/sera)</th>
<th>Mean antibody titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>96.8% (30/31)</td>
<td>94.5% (934/988)</td>
<td>6230</td>
</tr>
<tr>
<td>2011</td>
<td>100% (32/32)</td>
<td>78.9% (255/323)</td>
<td>5658</td>
</tr>
<tr>
<td>2012</td>
<td>93.3% (14/15)</td>
<td>77.4% (168/217)</td>
<td>3921</td>
</tr>
<tr>
<td>2013</td>
<td>100% (8/8)</td>
<td>85.4% (229/268)</td>
<td>4723</td>
</tr>
</tbody>
</table>

Poster Presentation
Conclusions: The results indicated that the pig sera showed apparent high positive antibodies to CSFV E2. In Thailand, attenuated CSF vaccines have been routinely used for the prevention and control of the disease (Damrongwatanapokin et al., 2006). Serological methods are valuable for monitoring and prevalence studies (OIE, 2008). The present results are monitoring the quantitative assessment of antibody levels of CSFV E2 in the eastern region of Thailand.

Keywords: classical swine fever, ELISA, eastern Thailand

References
Development of Method for Carbamate and Organophosphate Poisoning Diagnosis in Livestock Samples by pH Meter

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Introduction: Carbamate (CM) and organophosphate (OP) are a group of insecticides which have been extensively used in agriculture to protect crops from a variety of pests and can also be used for controlling external parasites in livestock. Misuse, accident and contamination can effect directly to livestock causing economic losses (Meerdink, 2003). Chromatography technique, the conventional method, for detection of insecticides is costly and complicated. Therefore, the objective of this study was to develop pH meter method based on cholinesterase inhibition technique to detect carbamate and organophosphate insecticides in livestock samples which was used to diagnose insecticides poisoning.

Methods: The samples including liver, stomach content and animal feed were detected by using pH meter method based on cholinesterase inhibition technique. They were extracted with acetonitrile followed by evaporation to dryness and dissolving in barbital phosphate buffer pH 8.4. Then, the extracted samples were performed to determine cholinesterase inhibition. Briefly, adding 5.0 of blood enzyme reagent and incubated at 37°C for 15 minutes. The pH (pH₁) of mixture of each sample was measured. After that, 0.1 ml of 7.5% acetylcholine iodide was added and incubated at 37°C for 20 minutes. At the end of the incubation period, the pH (pH₂) of mixture of each sample was also measured. Similarly, the blank sample was performed without the sample (Mohammad et al., 2007). The remaining cholinesterase indicated the level of carbamate and organophosphate insecticide residues in samples. For the positive samples consideration, cholinesterase inhibition in sample was compared to cholinesterase inhibition in blank sample to evaluate percentage of inhibition, which was higher than 50.0% cholinesterase inhibition (Kim et al., 2007). In order to determine the efficiency of developed method; 4 carbamate (carbofuran, 3-hydroxycarbofuran, methomyl and carbaryl) and 4 organophosphate (chlorpyrifos, dichlorvos, methy-parathion and prothiofos) were used as the representative of insecticides to evaluate detection limit. Total of 106 unknown samples were evaluated the performance of test by comparison between the developed method and high performance liquid chromatography (HPLC) method. The relative sensitivity, relative specificity, relative accuracy, relative positive predictive value and relative negative predictive value were calculated using the two by two table according to Eleftherios (2002). In addition, blood cholinesterase activity of animals including cows, cattle, goats, milk goats, sheep and elephants were analyzed based on electrometric method using pH meter for establishing a normal reference value.

Results: The results showed that the detection limit of 4 carbamate and 4 organophosphate insecticides that can be analyzed were ranging between 0.20-4.00 and 0.50-2.00 mg/kg, respectively. The comparison of results which was detected by this method and HPLC showed 88.9% relative sensitivity, 100.0% relative specificity, 98.1% relative accuracy, 100.0 % relative positive predictive value and 97.8% relative negative predictive value, which were in the acceptable levels. The normal level of blood cholinesterase activity of cows, cattle, goats, milk goats, sheep and elephants were ranging between 0.43-0.75 (DpH/20), 0.41-0.78 (DpH/20), 0.23-0.55 (DpH/40), 0.25-0.67 (DpH/40), 0.19-0.35 (DpH/40) and 0.09-0.23 (DpH/30), respectively.

Conclusions: pH meter method, based on cholinesterase inhibition has been developed for the determination of carbamate and organophosphate insecticides in livestock samples which can be used for poisoning diagnosis. This method is simple, time reduction, cost-effectiveness and can be done in the field.

Keywords: carbamate, organophosphate, cholinesterase inhibition, pH meter

References
Development of the Rapid Immunological Methods for Diagnosis of Leptospirosis in Livestock

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Introduction: Leptospirosis is the worldwide zoonotic disease caused by the Gram negative spirochete bacteria, *Leptospira* spp(1). This bacterium is secreted in urine of reservoir animal such as rat, cattle, pig, dog and infected to human via skin contacted(2). These leptospirosis animals not only the primary rout of human infection caused the public health problem, but the infection of livestock animal also very important. The infection of cattle, pig, and sheep resulted in economic outcome such as drop in daily product yield, fatal to young animal, failure of pregnancy(3) and cannot export to another country. The laboratory diagnosis of Leptospirosis is the detection of leptospira infection by both direct and indirect method such as bacterial culture, PCR, and antibodies testing. There are limitation problem of these methods including, long term culture, an expensive machine or an expert worker requirement. Even through the microscopic agglutination test (MAT) is the gold standard method for leptospira antibody detection, the leptospirosis animals have very low amount of antibody but high amount of *Leptospira* bacteria in urine. Therefore, we developed the immunochromatography(IC) strip test for diagnosis the *Leptospira*in livestock urine. This method is rapid and easily detection by naked eyes.

Methods: The specific monoclonal antibodies to *L. interrogans* serogroup were used in IC development. The rabbit serum (pAbs) and monoclonal antibody (mAbs) were purified by affinity protein G column. The IgG Ab were test with 23 serogroup of *L. interrogans* and known pooled positive and pooled negative urine. Pooled rabbit IgG specific to 23 serogroup of *L. interrogans* was conjugated with colloidal gold to generate the captor Ab on the conjugated pad.

Immunochromatography: The sample containing *Leptospira* Ag was captured by capture Ab on conjugated pad. The Ag-Ab complexed moved onto reaction membrane by capillary force and reacted with the broadly specific mAb on test line and control line.

Dot Blot Assay: We developed the dot-blot assay to detect the leptospira in livestock urine. The 25 positive and 100 negative urine samples which analyzed by PCR was used for evaluation of test. The pooled α-*Leptospira* pAbs was use to detected the dropped of urine on membrane. The signal of the test were developed after adding the alkaline phosphatase conjugated 2nd Abs and its substrate.
**Results:** Our result found that the lowest positive detection of strip test is 10 µg/ml (2µg) *Leptospira* and negative result with the other bacterial testing including *E. coli, S. aureus, Salmonella spp., Shigella spp.* except the *K. pneumonia* which the cross reactivity is weakly positive even in high amount of bacterial concentration (1mg/ml). Moreover, we use the strip test with the known 25 positive, 100 negative animal urine, and 30 urine sediments which are identified by PCR of *lipL32* gene.(4). This strip test cannot detect the *Leptospira* antigen in both urine and urine sediment. This result might from the low sensitivity or the loss of specificity of strip test to *Leptospira* antigen in urine specimen. Furthermore, we use the dot blot method by using the pooled rabbit IgG anti-leptospires Ab for detect the *Leptospira* antigen in urine sample. The 18 of 25 positive samples were positive and the 83 of 100 negative urine samples were negative when analyzed by dot-blot assay. The sensitivity, specificity, positive predictive value, and negative predictive value of the dot blot method compared with PCR method were 72%, 83%, 51.42%, and 92.22%, respectively.

**Conclusions:** We developed the IC strip test for detection of leptospira Ag in the sample. The laboratory results showed that the detection limited of the IC strip was 2 µg of *Leptospira* protein. The IC strip was no cross reaction with *E. coli, S. aureus, Salmonella spp., Shigella spp.* Even through the IC strip reacted with *K. pneumoniae*, the weakly positive result only occurred at high concentration (1mg/ml). The detection of leptospira Ag in positive live stock urine was limited. This might be from the uncontrollable condition in the sample. The 25 positive urine samples show weakly positive when analyzed by real time PCR of *lipL32* gene. Therefore, it was possible that there are too low concentration of leptospira protein to detection on IC strip. Furthermore some physical, chemical and biological factors in the urine sample including, the high pH, some proteins, bacterial and color substance might be interfering with the reaction.

We developed the dot-blot assay to evaluate the sensitivity and the specificity of our Abs in detection of leptospira in the urine. We showed that this method had a capacity to detect the leptospira Ag in livestock urine sample by the 72% sensitivity, 83 % specificity.

**References**


Introduction: Elephant endotheliotropic herpes virus (EEHV) is a severe cause of death in Asian elephant (*Elephas maximus*) worldwide. In calves, mortality rate is as high as 85% (Hayward, 2012). EEHV is normally detectable in dead elephant’s organs. However, we can also detect EEHV by trunk swab from healthy live Asian elephant. This study aimed to compare DNA sequences of EEHV obtained from dead and live elephants, and further try to identify other determinants of the disease.

Methods: Samples were extracted for genomic DNAs. The DNAs were further amplified using PCR method (Latimer et al., 2011) followed by direct sequencing for partial sequences of DNA polymerase gene. A phylogenetic tree was constructed using a neighbor-joining analysis of the partial sequences of DNA polymerase gene of our EEHV comparing with other related nucleotide sequences retrieved from Genbank database (Fig.1). Identity of the sequences was then compared by multiple nucleotide sequence alignment method (Table.1). Finally, a comparison of amino acid was performed (Fig.2).

Result: All 3 tested samples were categorized into EEHV1a (Fig.1). The percentage of their identities is between 98.90% - 99.50% (Table.1). Only one different variance was found among the 3 amino acid sequences. The difference was found in Case1051_tongue at amino acid position 67 (Fig.2).

<table>
<thead>
<tr>
<th>Sequences</th>
<th>JX011037.1_EEHV1A</th>
<th>Case651_tongue</th>
<th>Case1051_tongue</th>
<th>Trunk_swab</th>
</tr>
</thead>
<tbody>
<tr>
<td>JX011037.1_EEHV1A</td>
<td>99.50%</td>
<td></td>
<td>99.30%</td>
<td>99.10%</td>
</tr>
<tr>
<td>Case651_tongue</td>
<td></td>
<td>99.50%</td>
<td>99.30%</td>
<td>99.10%</td>
</tr>
<tr>
<td>Case1051_tongue</td>
<td>99.30%</td>
<td>99.30%</td>
<td>98.90%</td>
<td></td>
</tr>
<tr>
<td>Trunk_swab</td>
<td>99.10%</td>
<td>99.10%</td>
<td>98.90%</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1 Phylogenetic tree of EEHVs DNA polymerase. The tree was constructed using a neighbor-joining analysis. The number on each node indicates bootstrap value and the thick line represents group of EEHV1a.

Table 1 Matrix of Percent ages of Identities of EEHVs DNA polymerase nucleotide.

Figure 2 Multiple amino acid alignments of EEHVs DNA polymerase obtained from dead Asian elephantsamples (CASE651_tongue, CASE1051_tongue) and live Asian elephant (Trunk_swab) comparing with related sequences retrieved from Genbank database (JX011037.1_EEHV1a). Dots identify positions with identical amino acid residues.
Discussion and Conclusion: The present study revealed that all samples in this study were classified into EEHV1a which is a severe disease causing group (Ehlers et al., 2006). However, EEHV1a can be found also in asymptomatic elephant (Latimer et al., 2011; Hardman et al., 2012). Due to insufficiency of sample, it is not possible to conclude whether different position of amino acid is one of virulent factors. However, according to veterinary research and development center (lower northeastern region), all elephants died due to EEHV infection in the region aged only between 1-3 years. Our study was in line with Sripiboon et al.,(2013) that found EEHV1A in a 2-year-old calve and EEHV4 in another 4-year-old calve. Thus, one potential factor could be the age of infected elephants and might be also their weaning period. Further studies on the effects of the immune system of Asian elephant, weaning period and viral infectivity are needed before any conclusions can be reached.

Keywords: Sequences analysis, Elephant endotheliotropic herpes virus, tongue, trunk swab, Asian elephant

References


Genetic Analysis of Cricket Paralysis Virus: The Emerging Disease of House Cricket in Thailand

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Introduction: Cricket paralysis virus (CrPV), a single strand positive-sense RNA (Eaton and Steacie, 1980), was first isolated in 1970 by Carl Reinganum. At present, CrPV is classified as a member of an Order Piconavirales, Family: Dicistroviridae and Genus: Cripavirus by International Committee on the Taxonomy of Viruses (ICTV) in 2012. CrPV caused paralytic disease in house cricket in many countries. The crickets flipped over their back, paralyzed and died. The aim of this work was to diagnose the cricket paralysis virus infection in house cricket in Thailand.

Materials and Methods: House crickets (Acheta domestica, Acheta testacea and Gryllus bimaculatus) in the farms in some provinces of Thailand showed the signs of sluggish, sporadic twitching of antennae, partially paralyzed in the hind legs and died. The causing paralysis was suspected to be of CrPV which resulting in the loss of 95% of batch. Fresh dead crickets which showed clinical signs were collected and the whole body of cricket was extracted for viral RNA using the commercial kit. RT-PCR was performed for noncoding region (NCR), intergenic region (IGR) and RNA dependent RNA polymerase (RdRp) of the CrPV genome (Wilson et al., 2000; Baker and Schroeder, 2008). The PCR products were ligated and transformed into the plasmid vector and E. coli, respectively and subsequently sequenced. The sequences were analyzed by BLAST program and phylogenetic analyses among the products and related viruses were conducted by the neighbor-joining method using MEGA5.

Results: The amplified products of NCR, IGR and RdRp were obtained at expected sizes of 705 bp, 207 bp and 1.2 kb and their nucleotide sequences showed 83%, 88% and 90% similarity to those of CrPV (NC003924), respectively. The phylogenetic tree of the nucleotide sequence of IGR among the related virus was shown in Fig 1. In addition, the amino acid sequence of the non-structural protein encoded by RdRp also had 94% similarity to that of CrPV. The alignment and the phylogenetic tree of amino acid sequences of the nonstructural protein of the related viruses were shown in Fig 2 and Fig 3, respectively.

Conclusion: The results indicated that the viral RNA found in house crickets was identified as CrPV a cause of paralysis and death in the cricket farms. This is the first report of CrPV which is the emerging disease of house cricket in Thailand. We concluded that RT-PCR was a suitable tool and provided an appropriate data for diagnosis of CrPV infection. However, CrPV is distributed worldwide, monitoring of the disease and further analysis of the whole genome of the viruses in different regions may clarify diversity of the CrPV and the epidemiology of the disease in Thailand.

**Fig 1** Phylogenetic analysis of nucleotide sequences of the IGR of the cases and member of Dicistroviridae including CrPV, Drosophila C virus (DCV), Black queen cell virus (BQCV) and Aphid lethal paralysis virus (ALPV).
Fig 2. Alignment of the amino acid sequences of RdRp encoded protein of the case and CrPV (AAF80998)

Fig 3 Phylogenetic analysis of amino acid sequences of RdRp encoded protein of the cases and member of Dicistroviridae including CrPV, Drosophila C virus (DCV), Aphid lethal paralysis virus (ALPV) and Rhopalosiphum padi virus (RhPV).

Keywords: genetic analysis, emerging, cricket paralysis virus, house cricket, paralysis, twitch

References
Seroprevalence of Paratuberculosis in Dairy Cattle in the Upper Northeastern Region of Thailand by Complement Fixation Test and Fecal Smear

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Introduction: Paratuberculosis or Johne’s disease is a chronic contagious disease that affects ruminant animals such as cow, buffalo, goat and sheep. In cattle, the common clinical signs of paratuberculosis are diarrhea, weight loss, intermandibular edema. The infected animals become increasingly emaciated and usually die from dehydration (Kanameda and Ekgatat., 1993). This disease can be transmitted through ingestion of agent in the feces from infected animal. Regards long incubation period of agent in carrier animal that will release agent in the environment and spread to other animal. Detection of antibodies against Mycobacterium avium subsp. Paratuberculosis using Agar gel immunodiffusion test (AGID) (Shermann et al., 1984), Immunoelectrophoresis, Fluorescent antibody test (FAT), Complement fixation test (CFT) (Sockett et al., 1992), ELISA (Yokomizo et al., 1991). For colony detection in fecal smear using Acid fast staining (Ziehl-Neelsen). Finally, culling the positive animals are the way to control and eradicate the disease.

The aims of this study are to monitor the seroprevalence of paratuberculosis in dairy cattle in the Upper Northeastern Region of Thailand using complement fixation test and to compare this test with fecal smear to find out the agreement of the tests.

Methods:
Serum Samples: Field samples were sera from 46,395 dairy cattle (2,666 farms in the upper northeastern part of Thailand) and collected during the year 2010 to 2012.
Fecal Sample: Field samples were 438 fecal samples (from CFT positive 119 samples and CFT negative 319 sample) which collected from dairy cattle farms in the upper northeastern Thailand during 2010 – 2012.
Serological tests: The sera were tested for antibody against Mycobacterium avium subsp. paratuberculosis by CFT (Standard Guide for Laboratory Animal of the National Institutes of Health, 2543).
Fecal test: The fecal samples were examined by Acid fast staining as described by Morris et. al (1993):
Analytical: The serological tests were analyzed by descriptive statistics (percent positive in individual and farm level). Compare the results of CFT and fecal smear (Acid Fast staining) by using statistic McNemar Chi-Square for the Kappa value (Cohen and Weighted, 1968)

“Kappa statistic” K = Pr(a) - Pr(e) / 1 - Pr(e)

Results: The overall result for antibody against Mycobacterium avium subsp. paratuberculosis from dairy cattle sera by CFT, revealed that 0.99% and 13.05% of the samples were positive by individual and farm level respectively. During the years 2010 to 2012 there were 1.65%, 0.89% and 0.49% positive by individual level and 16.73%, 13.90% and 8.13% by farm level, respectively. The respectively high sero-positive by individual level was found at Kalasin (4.10%), Sakonnakhon (1.47%) and Nongbaulamphu (1.22%) whereas by farm level was found at Kalasin (26.92%), Loei (23.08%) and Nongbualamphu (19.35%). From the result of M. paratuberculosis detection by fecal smear (acid fast), there were 56.30% and 5.33% M. paratuberculosis detected from CFT positive and negative animals, respectively. The agreement between the tests with a Kappa value was 0.55.

Conclusion: The prevalence of Paratuberculosis parameters by CFT during 2553 – 2555 were 1.65%, 0.89% and 0.49%, respectively (the average was 0.99%). Because of this disease is asymptomatic and can spread to other animal (McIntyre and Selmen, 1981), so it quite difficult to control. However, the trend of the disease prevalence was decreased respectively in each year, in particular the prevalence in farm were found 16.73%, 13.90% and 8.13%. It showed that they have been in good disease control by the Department of Livestock Development via a disease-free farms campaign under governmental policy since 2546. When compare the results by fecal smear (Acid fast) with CFT, we found that the results of M. paratuberculosis detected by fecal smear (acid fast) were 56.30% and 5.33% (from CFT positive and negative animals, respectively). The agreement between the tests with a Kappa statistic was 0.55, it showed that the two approaches were moderate agreement, so they could be used. We can tested Paratuberculosis by CFT together with a Fecal smear (acid fast).

This report shows that Paratuberculosis was still found in dairy cattle in the Upper Northeastern region. It should be continuously and keep on the appropriate control program, as well as to educate and promote animal health to support prevention and control more effectively.

Keywords: Paratuberculosis, complement fixation test, fecal smear, acid fast.
References


Monitoring of Chicken Infectious Anemia in the Risked Broiler Farms

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Introduction: Chicken infectious anemia (CIA) caused by Chicken anemia virus (CAV) causes economic loss problem in chicken industry worldwide. This virus is important because of transovarian transmission (Schat and Van Santen, 2008) and inducing immunosuppression (Hailemariam et al., 2008). This research is to investigate the causes for outbreak of CAV infection in two broiler farms in Lopburi and Saraburi province in 2008-2010 to prevent the repeated outbreak and follow the trend of disease in these farms.

Methods: Forty-one carcasses of chicken were sent for CAV infection diagnosis at NIAH. The birds were necropsied and samples of thymus, liver, and spleen were collected for viral investigation. The homogenized suspension of pooled tissues was analyzed by a polymerase chain reaction (PCR) following DNA extraction by QIAamp DNA Mini kit (QIAGEN). One hundred and thirty eight tissue samples were collected from chicken in both farms for CAV detection every 3 months before and after changed the chick provider and breeders. Eighty blood samples and 4 carcasses were collected from breeder in chick provided farms after CAV detection in broiler farms. DNeasy blood & tissue kit (QIAGEN) was used for DNA extraction from blood sample followed by PCR. After culling all old breeders, the blood was collected every three months until the study finished.

Result: The chicken carcasses from both broiler farms showed emaciation, pale muscles, yellowish bone marrow, hemorrhage in breasts and legs, spleen and thymus atrophy. CAV was detected by PCR technique from pooled organs of 10 and 31 chickens in two broiler farms, respectively. The size of PCR product was shown around 419 nucleotides (Rath et al., 2003) by ethidium bromide agarose gel electrophoresis. The samples were collected every 3 months until the end of this study. Thirty-two samples from broiler had been found CAV positive before changed the chick provider and breeders. Moreover, CAV infection was found from all 80 blood samples and 4 carcasses of breeders by PCR. After changed the chick provider and breeders, CAV was not found from 106 carcasses from chicken in both broiler farms until ended. Additionally, 160 blood samples from breeder showed negative results in CAV infection after eliminated the old breeders in the farm.

Table 1 Positive results of CAV from chicken organs by PCR in each trimester in 2008-2010

<table>
<thead>
<tr>
<th>Farm</th>
<th>Positive samples/Total samples</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm 1</td>
<td>10/10</td>
<td>15/15</td>
</tr>
<tr>
<td>Farm 2</td>
<td>31/31</td>
<td>0/3</td>
</tr>
<tr>
<td>Farm 3</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: - = no sample  * = blood sample

Conclusion: The outbreak of CAV in two broiler farms was investigated in this study. Broiler was provided by the same breeder farm. After CAV detection continuously in spite of good management in broiler farm, the breeder farm was suspected for the cause of viral transmission. These farms were monitored for CAV infection every three months by carcass sampling in broiler farms and blood sampling in breeder. All old breeders were eliminated following CAV detection in blood samples. After changing the chicken provider and removed the old infected breeder, CAV was not detected in all samples from these farms until ended.

Keywords: Chicken Infectious Anemia (CIA), Chicken Anemia Virus (CAV), broiler

References
National Institute of Animal Health (NIAH) is the main authority responsible for animal health of Thailand that assured by international standard and designated as the reference center of South East Asia. NIAH implements a quality system of ISO/IEC 17025 : 2005 (General requirements for the competence of testing and calibration laboratories) that aim to (1) promote professional management of the laboratories in respect of analysis, test, and diagnosis of animal health, (2) yield qualified and accurate results, (3) achieve ultimate customer satisfaction, (4) improve our competency progressively with international acclaim, (5) facilitate exports of livestock products.

In present, NIAH and cooperative laboratories have been accepted as accredited laboratories complying with the ISO/IEC 17025 : 2005 from the Bureau of Laboratory Quality Standards, Ministry of Public Health. Among 96 specific tests accredited, seven of these have been harmonized uniformly to operate in the national level including:

1. Detection of rabies virus antigen in brain tissue using Fluorescent Antibody Technique.
2. Detection of antibody against H5 Avian Influenza viruses by Haemagglutination Inhibition (HI).
3. Diagnosis of Avian Influenza and Newcastle disease by viral isolation in embryonated eggs and Haemaglutination (HA) - Haemagglutination Inhibition(HI).
4. Diagnosis of Avian Influenza and Newcastle disease by viral isolation in cell culture and Haemaglutination (HA) - Haemagglutination Inhibition(HI).
5. Detection of antibody against 3ABC non-structural protein of Food and Mouth Disease (FMD) virus by ELISA.
6. Detection of antibody against *Brucella abortus* in bovine serum by Rose bengal test.
7. Detection of antibody against *Brucella abortus* in bovine serum by Complement fixation test.

Additionally, NIAH has performed biosafety and biosecurity program to ensure the safety of laboratory workers involved with microbes, chemicals, submitted samples, laboratory animals, as well as to prevent the spread of these contaminants in the environments. In order to develop the program, we obtained helpful references and guidance from international organizations such as World Health Organization (WHO) and United States Centers for Disease Control and Prevention (CDC) for compilation of Standard Operative Procedures (SOPs) for laboratories. A working group of biosafety and biosecurity has been formed to take charge of the SOPs arrangement, providing organized systems for laboratory procedures, initiating training courses, and performance appraisal related to laboratory biosafety and biosecurity.
From the past to present: Electronmicrograph Demonstration of Veterinary Pathogenic Agent Isolation in Pathology Section, NIAH Thailand

Somchit Ruchikuan  Sontana Mimapan  Tuangthong Patchimasiri  and Ladda Trongwongsa

**Avian**

- **Chicken:** Reovirus (Cell culture)
- **Chicken:** Adenovirus (Cell culture)
- **Chicken:** IBDV (Bursa of Fabricius)
- **Chicken:** Marek’s disease virus (Cell culture)
- **Chicken:** Chicken poxvirus (CAM)
- **Chicken:** Plasmodium gallinaceum (Liver)
- **Chicken:** Trichomonas spp. (Intestine)
- **Chicken:** NDV Immunogold technique (Cell culture)
- **Chicken:** AlV Immunogold technique (Cell culture)
- **Goose:** Parovirus (negative stain)
- **Goose:** Cryptosporidium spp. (Bursa of Fabricius)
- **Goose:** Duck viral enteritis (Esophagus)
- **Duck:** Duck poxvirus (CAM)
- **Pigeon:** Avipoxvirus (Skin)
- **Pigeon:** Herpes virus (Liver)
- **Pigeon:** ILTV (Cell culture)
- **Common Myna:** Atoxoplasma spp. (Liver)
- **Macaw:** Avian polyomavirus (Kidney)
From the past to present: Electronmicrograph Demonstration of Veterinary Pathogenic Agent Isolation in Pathology Section, NIAH Thailand

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Swine

Swine: Circovirus (Lymph node)
Swine: ADV (Fetus)
Swine: Swine poxvirus (Skin)
Swine: PRRS virus (Lung)
Swine: Coronavirus (Cell culture)
Swine: Trypanosoma evansi (Blood)
Swine: E. coli (Intestine)
Swine: E. coli (Negative stain)
Swine: Treponema spp. (Negative stain)

Others

Cattle: Babesia spp. (Liver)
Cattle: FMD (Negative stain)
Sheep: Pulmonary adenomatosis multilamellate bodies (Lung)
Cricket: Iridovirus (Adipose tissue)
Title: The 3rd Thailand-Japan Joint Conference on Animal Health 2014
Editor: National Institute of Animal Health, Department of Livestock Development, Kasetklang, Chatuchak, Bangkok 10900, Thailand
First edition, 2014
Published by National Institute of Animal Health, Department of Livestock Development, Kasetklang, Chatuchak, Bangkok 10900, Thailand
Printed by The Agricultural Co-operative Federation of Thailand., Limited.
Chatuchak, Bangkok 10900, Thailand