10th year anniversary of

Veterinary Public Health Centre for Asia Pacific

- Alumni meeting on "ASEAN: Stepping forward into One Health Society"
- "The 3rd Food Safety and Zoonoses Symposium for Asia Pacific"
- "The 1st Regional EcoHealth (EH) Symposium: Social and Environmental Dynamic on Human and Animal Health"

10th Year Anniversary of Veterinary Public Health Centre for Asia Pacific

10th Year Anniversary of Veterinary Public Health Centre for Asia Pacific

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Welcome Messages

Assoc. Prof. Dr. Lertrak Srikitjakarn
Dean
Faculty of Veterinary Medicine
Chiang Mai University
THAILAND

Warmest Welcome to the symposium marking the 10th Year Anniversary of VPHCAP, Faculty of Veterinary Medicine, Chiang Mai University!

It's not every year that an organization can celebrate 10 years of service, innovation and dedication -- and this celebration by VPHCAP is indeed special. Since VPHCAP founding in 2003 with a vision of international academic and collaborative (training) centre in the Veterinary Public Health including food safety, zoonoses and international trading of animal products. Our alumni, faculty, students and staff have been working for the betterment of animals, people and our environmental health. As such, we recognize the importance of imparting our academic accomplishments to the local and greater communities. To celebrate the pass success of the VPHCAP, The symposium is organized base on local and international academic activities to generate innovative knowledge, exchange experiences and network communication between expert and academic scientists in food safety and zoonoses, EcoHealth/OneHealth for further academic cooperation and researches.

We are exceptionally proud of all of our accomplishments, and I am excited about what the future holds for VPHCAP.

Thank you for taking a moment to catch up on all the good work the VPHCAP is doing.

Warmly,

Lertrak Srikitjakarn
Dean
Faculty of Veterinary Medicine
Chiang Mai University
THAILAND

Welcome Messages

Assist. Prof. Khwanchai Kreausukon
Director
Veterinary Public Health Centre for Asia Pacific
Faculty of Veterinary Medicine
Chiang Mai University
THAILAND

It is such a great honor and privilege for me to extend our greeting for **"The 10th Anniversary of Veterinary Public Health Centre for Asia Pacific"** symposium. This symposium is truly an essential academic cooperation for our field. As our world has increasingly been globalized, the emerging of infectious and zoonotic disease has also been spreading rapidly through our globe as well. That means that the roles of veterinarians would become much more demanding than previous years. The increasing of roles has shifted our professional to a new paradigm – since our responsibilities have become much wider with the aim of solving numerous health issues.

Acknowledging of such issues, the Veterinary Public Health Centre for Asia Pacific (VPHCAP) was established in 2003, under the branch of Faculty of Veterinary Medicine, Chiang Mai University. Our main goals have always been to serve an international academic and collaborative centre in the fields of food safety, zoonoses and epidemiology.

Master in Veterinary Public Health (MVPH) program was established by cooperation between us and Faculty of Veterinary Medicine, Freie Universitaet Berlin, Germany. Since then, we have 4 generations of alumni who have been contributing significantly to our field. We genuinely realize our program would not have been as much successful; without the credibility and recognition of Freie Universitaet.

This symposium is not only to celebrate our anniversary, it is organized to illustrate our past success and stimulate innovative knowledge. This special event has been conducted with great efforts of our directors, professors, staffs and supporters.

On the behalf of The Veterinary Public Health Centre for Asia Pacific, Faculty of Veterinary Medicine, Chiang Mai University and the organizing committee; we welcome you to "**The 10th Anniversary of Veterinary Public Health Centre for Asia Pacific."**

As much as I hope this symposium will contribute to the improving of our beloved field; I also hope such occasion will strengthen relationships among us, stakeholders, alumni, current students and all participants – professionally and personally.

Finally, I truly hope that this symposium will meet up to your expectation.
I also wish you will enjoy your time in Chiang Mai and please with our sincere hospitality.

Sincerely,

Khwanchai Kreausukon
Director
Veterinary Public Health Centre for Asia Pacific
Faculty of Veterinary Medicine
Chiang Mai University
THAILAND

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International Symposium on 10th Year Anniversary of Veterinary Public Health Centre for Asia Pacific

ASEAN stepping forward into One Health Society
Tuesday 2 July 2013, The Imperial Mae Ping Hotel, Chiang Mai

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08.30-09.00	Registration
09.00-09.20	**Opening ceremony:** • *Remark address by Assist. Prof. Dr. Khwanchai Kreausukon* • *Opening speech by Assoc. Prof. Dr. Lertrak Srikitjakarn* *Moderator: Dr. Tongkorn Meeyam*
09.20-10.00	**Welcome remarks:** • *DAAD representative* • *FUB representative by Prof. Dr. Reinhard Fries* • *Alumni representative by Dr. Warangkhana Chaisaowong*
10.00-10.15	**Introduction for Alumni activities** *Moderator: Dr. Maximilian Baumann*
10.15-10.45	Coffee Break
10.45-12.00	**Alumni Meeting: Group Activity I** *Moderator: Dr. Maximilian Baumann* *Dr. Tongkorn Meeyam*
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15.00-15.30	Coffee Break
15.30-16.30	**Summary** *Moderator: Dr. Maximilian Baumann*
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International Symposium on 10th Year Anniversary of Veterinary Public Health Centre for Asia Pacific

The 3rd Food Safety and Zoonoses Symposium for Asia Pacific
Wednesday 3 July 2013, The Imperial Mae Ping Hotel, Chiang Mai

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International Symposium on 10th Year Anniversary of Veterinary Public Health Centre for Asia Pacific

The 3rd Food Safety and Zoonoses Symposium for Asia Pacific
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International Symposium on 10th Year Anniversary of Veterinary Public Health Centre for Asia Pacific

The 3rd Food Safety and Zoonoses Symposium for Asia Pacific
Thursday 4 July 2013, The Imperial Mae Ping Hotel, Chiang Mai

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	Session: An Update on Antimicrobial resistance *Chairman: Dr. Maximilian Baumann*				
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	Session: Ecosystem approach related to human health *Chairman: Dr. Jeffrey Gilbert*		14.00-14.20	Influence of Vaccine Cold Chain Handling on the Protective Rate of Rabies Post Vaccination of Dogs in Western Sumatra *by Heru Susetya*	E-01 120

International Symposium on 10th Year Anniversary of Veterinary Public Health Centre for Asia Pacific

The 3rd Food Safety and Zoonoses Symposium for Asia Pacific
Thursday 4 July 2013, The Imperial Mae Ping Hotel, Chiang Mai

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International Symposium on 10th Year Anniversary of Veterinary Public Health Centre for Asia Pacific

The 3rd Food Safety and Zoonoses Symposium for Asia Pacific
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International Symposium on 10ᵗʰ Year Anniversary of Veterinary Public Health Centre for Asia Pacific

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International Symposium on 10th Year Anniversary of Veterinary Public Health Centre for Asia Pacific

The 1st Regional EcoHealth (EH) Symposium: Social and Environmental Dynamic on Human and Animal Health
Saturday 6 July 2013, The Imperial Mae Ping Hotel, Chiang Mai

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	Session: EcoHealth approach for better management of zoonotic disease and human health *Chairman: Assist. Prof. Dr. Sumalee Lirtmunlikaporn*		
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INVITED PRESENTATIONS

Highly Pathogenic Avian Influenza (HPAI) – Lessons Learnt for Regional Perspective for Veterinary Public Health

Dr. Teruhida Fujita
Japan Livestock Technology Association (JLTA), Japan and FAO Representative

SUMMARY

Highly Pathogenic Avian Influenza (HPAI; H5N1) emerged in Asia in late 2003. The disease is of emerging/transboundary nature and was rapidly and widely spread to other continents including European, Middle East and African regions. The viruses of the disease have still been persistent and circulated among animals in the world, even after the first case reported for a long time ago. This zoonotic disease has posed serious and negative impacts on animal and human health and high-economic and social negative consequences. During the occurrences of HPAI in the Asian Region, weaknesses and strengths of veterinary services have been identified and learned, which included the importance and necessity of strengthening veterinary services, transparent and timely notification of disease cases, capacity building for accurate diagnosis and surveillance, rapid disease responses and preparedness, disease control at risk (at farm and live animal market levels)to prevent transmission of the disease among animals and even to humans, effective communication and awareness on the disease as well as good partnership between animal and human health sectors, establishment of international/regional networks and improved veterinary service delivery and education. Various international and regional activities including the support to technical guidance and capacity building and to regional networks/alliances have been taken in order to tackle HPAI and reduce the disease risks. Roles of veterinary public health are increasingly important and significant to control zoonotic diseases including HPAI. It is expected that VPHCAP, Chiang Mai University will be further sustainably developed and contribute a lot to the strengthening and improving of Veterinary Public Health in the Region.

BIOGRAPHY

Dr. Teruhida Fujita
Japan Livestock Technology Association (JLTA),
Japan and FAO Representative

Dr. Teruhida Fujita is the adviser of Japan Livestock Technology Association (JLTA), Tokyo, Japan. He was a former Chief Veterinary Officer of Japan, Ministry of Agriculture, Forestry and Fisheries (MAFF), Tokyo, a former Director of Animal Health & Production Division, FAO, Rome, Italy, a former OIE Regional Representative for Asia and the Pacific, Paris, France (based in Tokyo), a former Board Member of International Livestock Research Institute (ILRI), Nairobi, Kenya (based in Tokyo), and a former Visiting Lecturer of Tokyo University of Agriculture and Technology, Tokyo.

The People Centered Approach for Animal Food Systems

Prof. Dr. Karl-Hans Zessin
Faculty of Veterinary Medicine, Freie Universität Berlin, Germany

SUMMARY

Two characteristics of future animal agriculture globally are that consumption of livestock products is growing faster than world population and that the major increase in total production and consumption will happen in developing countries. Options to expand production onto new land no longer exist. Intensive production in 'industrial units' will take the majority of the production increase. Smallholders, of which 87% (550 million) are located in Asia-Pacific and potentially being the second resource, are at risk of being excluded from the livestock sector development. Policy attempts to transform the smallholder sector and make sizeable numbers of smallholders climb the 'livestock ladder' have been largely unsuccessful because of their overwhelming categorical, technocratic/ technical orientation. Little considered are that smallholder systems are highly heterogeneous and that food systems are about people. Food systems feed people, employ people and give people business opportunities. To make the food systems function, requires these people to make short and long term decisions. To understand the food systems, the 'milieu' in which smallholders behave in assessing impacts and options at the household and the systems level, effective policies and interventions must consider compliance incentives, cultures and livelihood issues of targeted smallholders. Some of it is dictated by economic production parameters of both factor and product markets, by institutional environments (rules, official and informal, and their enforcement) and by social, cultural and psychological factors. Presented are components for high resolution studies of a suggested multi-disciplinary smallholder project, starting at CMU in Thailand, which expands the 'people centered approach' demanded by Rushton et al. (2013) at PMAC.

BIOGRAPHY

Prof. Dr. Karl-Hans Zessin
Faculty of Veterinary Medicine, Freie Universität Berlin, Germany
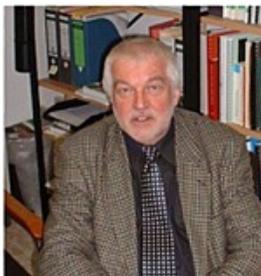
A veterinary graduate (Berlin, 1975), project manager of technical cooperation projects (Sudan: 1977-1982; Somalia: 1984-1988). MPVM (1983) and PhD (1991) of University of California, Davis. 1992 appointed as Professor in Veterinary Epidemiology at Freie Universität Berlin. Specialisation in teaching (university, various international user groups) and research on Veterinary Public Health. Initiator and course director of international Joint-Degree Master Programme in Veterinary Public Health with Chiang Mai University, Thailand. Retired from Freie Universität Berlin in October 2012.

Addressing Food Safety and Zoonoses: Opportunities for One Health Leadership

Prof. Dr. William Hueston, DVM, PhD
Executive Director, Global Initiative for Food Systems Leadership

SUMMARY

Food safety and zoonoses represent increasingly complex challenges as people, animals and food move around the world. Effectively managing the associated risks requires new forms of public-private-academic partnership where experts from many different disciplines work with the food industry and government officials. While technology provides new tools for detection and processing, creating a flexible animal and public health system to assure safe food and detect and respond to zoonoses is not a problem that can be 'solved' by a simple technical solution. Addressing food safety and zoonoses requires a new type of leadership where responsibility and authority are shared and adaptation to constantly changing conditions is the norm, not the exception.

Leadership is a process for catalyzing change. In the context of One Health, leadership is a shared responsibility rather than a title of authority. One Health leadership is unique in the wide range of disciplinary expertise that is needed to be effective. Each discipline brings a unique perspective. For example, a high functioning One Health leadership team working in food safety might include a food scientist, public health expert, surveillance nurse, ecologist, engineer and animal disease expert. Together they can generate a trans-disciplinary approach, one that transcends any individual discipline and uses systems thinking to better understand the situation.

One Health leadership requires exemplary communication skills, the ability to work across boundaries and disciplines, strategic thinking, influence skills and the ability to get results. All of these skills can be taught and must be incorporated into university curricula, on-the-job training and professional development courses. One Health leadership is critical in order to achieve incremental improvement for the grand challenges of global food safety and zoonoses prevention and control.

BIOGRAPHY

Prof. Dr. William Hueston, DVM, PhD
Executive Director, Global Initiative for Food Systems Leadership

Will Hueston directs Global Initiative for Food Systems Leadership for the Center for Animal Health and Food Safety at the University of Minnesota. His work focuses on One Health capacity-building, public policy and risk communication with the ultimate goal of creating integrated and harmonized systems for global food security. He has extensive experience facilitating strategic public-private partnerships to address the complex challenges and create exciting opportunities related to the global food system and public health.

Dr. Hueston has worked in private veterinary practice and held positions with a farmer-owned cooperative, the US and UK government, and four different veterinary colleges in the US. He also serves as Director of the University of Minnesota Food Policy Research Center and the World Organisation for Animal Health (OIE) Collaborating Center in Veterinary Services Capacity Building. Dr. Hueston holds faculty appointments in the College of Veterinary Medicine and the School of Public Health.

New Developments in Diagnostic Tools-opportunities for Food Safety and Outbreak Investigations

Prof. Dr. Thomas Alter
University Professor, Institute of Food Hygiene, Faculty of Veterinary Medicine, Freie Universität Berlin, Germany

SUMMARY

In the last years, novel diagnostic methods have more and more supported or replaced classical detection methods in food safety. Especially DNA sequence based methods are applied. With these methods, a fast detection of e.g. foodborne pathogens or GMO is possible. In addition, a quantification of these agents can be achieved. PCR based methods can be used to further characterize microorganisms in food (detection of virulence factors, antimicrobial resistance markers, stress response).Genotyping enables us to carry out outbreak investigations by comparing bacterial strains from different sources or to characterize bacterial populations in complex habitats.

Still, some aspects have to be solved:

-   How to distinguish viable from dead bacterial cells esp. in processed food?
-   The role of viable but not culturable bacterial cells;
-   Standardization of genotyping methods to enable a comparison of strains from different regions;
-   Bioinformatical analysis.

BIOGRAPHY

Prof. Dr. Thomas Alter
University Professor, Institute of Food Hygiene,
Faculty of Veterinary Medicine, Freie Universität Berlin, Germany

Thomas Alter studied veterinary medicine in Leipzig, Germany, and Dublin, Ireland. He received his Doctoral Degree in Food Microbiology from the University of Leipzig. After working as senior scientist in Leipzig and Zurich, Switzerland, he became Head of the National Reference Laboratory for *Campylobacter* and Deputy Head of the National Reference Laboratory for *Listeria monocytogenes* at the Federal Institute for Risk Assessment, Berlin, Germany. Since 2009 he is Managing Director of the Institute of Food Hygiene, Department of Veterinary Medicine, Freie Universität Berlin.

Zoonosis, Urinary Tract Infection, Uropathogenic *Escherichia coli*

Prof. Dr. Hisao Kurazono
Department of Animal and Food Hygiene, Obihiro University, Hokkaido, Japan

SUMMARY

Escherichia coli is a major causative agent of uncomplicated urinary tract infection (UTI). The *E. coli* strains isolated in UTI are designated as uropathogenic *E. coli* (UPEC). UTI occurs not only among humans, but is also observed in companion animals such as dogs and cats.

Seven virulence factors have been documented to play an important role in causing human UTI. In addition, it is known that several serotypes are preferentially associated with human UPEC strains. Recently, it has been found that each of the uropathogenic virulence factors is significantly associated with these serotypes. The characteristics of UPEC strains from humans are similar to those of UPEC strains isolated from companion-animal UTI. These findings suggest that similar *E. coli* strains might be able to cause infection in humans and companion animals.

We have previously identified a putative pathogenicity island (PI), which was more frequently found in UPEC strains than in *E. coli* strains isolated from healthy individuals. The PI contains auropathogenic specific protein gene (*usp*) and three small open reading frames (open reading frame unknown: *ofrU1-3*). We demonstrated that Usp significantly enhanced the infectivity of *E. coli,* whereas the three small proteins downstream of *usp* failed to exhibit this effect in a mouse urinary tract infection model. Further, we have demonstrated that the distribution of *usp* in UPEC strains isolated from companion animals was not only more frequent than those of other urovirulence factors, but also was associated with common serotypes of UPEC strains that cause human UTI. According to our findings, we presume that dogs and cats are alternative reservoirs for Human UTI. A homology search revealed that Usp and OrfUs have a homology with nuclease-type bacteriocins and immunity proteins respectively. However, the molecular activity of these proteins was never investigated. In this presentation, I also show that Usp is a new member of the H-N-H superfamily.

BIOGRAPHY

Prof. Dr. Hisao Kurazono
Department of Animal and Food Hygiene, Obihiro University, Hokkaido, Japan

Hisao Kurazono was educated at Nihon Veterinary and Zoo Technical University (BVS) and Osaka Prefecture University (MS & PhD: Veterinary Public Health). After a postdoc in Germany and the U.S.A., he successively held positions at Colleges of Medicine in Tokyo University, Kyoto University and Tsukuba University. He joined the faculty of Okayama University College of Medicine in 1999, having achieved the rank of full professor. Thereafter, he was promoted as a professor of Osaka Prefecture University (Veterinary Public Health) and, later Obihiro University of Agriculture and Veterinary Medicine (Animal and Food Hygiene). Currently, he is the vice president of the University. He is also involved in teaching veterinary students (Microbiology and Veterinary Public Health) and graduate students (Microbiology and Food Hygiene).

Veterinary Public Health Education and Training

Dr. Karin Hamilton
University of Minnesota, College of Veterinary Medicine, USA

SUMMARY

Public health is an important area of veterinary medicine that deserves attention, especially in veterinary school curricula in the United States, where the majority of veterinary students become small animal veterinarians. The field of veterinary public health (VPH) recognizes the undeniable connection between humans, animals, and the environment often focusing on food security and zoonotic diseases, with an emphasis on prevention at the population level. The University of Minnesota (UMN), College of Veterinary Medicine currently offers four VPH education or training courses/programs: a one week core course for all veterinary students, a one week elective for veterinary students interested in public health, a four year dual degree program (DVM/MPH) for veterinary students interested in public health, and a two year residency program for mid-career veterinarians. The VPH residency is the first of its kind in the United States and provides practical training and experience to veterinarians while receiving mentorship. Graduates from this program have gained employment with a variety of organizations including the US Centers for Disease Control and Prevention, the University of Minnesota, the National Pork Board, and the US Department of Agriculture. Through international collaborations with UMN, other universities around the world such as Makerere University in Uganda and Chiang Mai University in Thailand are starting new public health training programs including a VPH Residency, a One Health Residency, and an international MPH program.

BIOGRAPHY

Dr. Karin Hamilton
University of Minnesota, College of Veterinary Medicine, USA

Dr. Karin Hamilton is public health veterinarian at the University of Minnesota, College of Veterinary Medicine in the USA. She earned her Doctorate of Veterinary Medicine at Tufts University. After completing a Veterinary Public Health Residency program and earning a Master of Public Health at the University of Minnesota, she became boarded by the American College of Veterinary Preventive Medicine. Prior to working at the University of Minnesota, Dr. Hamilton served as a veterinary officer with the US Army, where she worked in Africa, the Middle East, and Asia, in addition to the US. For the past two years, she has been working primarily with Southeast Asian universities on a US Agency for International Development funded project called RESPOND, which focuses on building public health capacity in tropical areas around the world.

Keeping Food Chains Safe: Strategies of Control and Prevention

Prof. Dr. Reinhard Fries

University Professor, Institute of Meat Hygiene and Technology, Faculty of Veterinary Medicine, Freie Universität Berlin, Germany and Head of FAO Reference Center for Veterinary Public Health

SUMMARY

Each food commodity is characterised by specific background (food chain) and hazards. This applies also for global food chains and for different geographic regions, depending on their internal structure and organisation. We observe also global movement of animals and global trade with foods of all origin. Hence, food chains transfer risks globally, and there is a general need for identification and control of chain- and region- specific risks, which applies for human health, possibly also animal health or items of animal wellbeing. Having decided on such targets on a scientific base, detection techniques should be listed first and subsequently assessed for their specific purpose, under the light of precision, availability, of personal resources and of the prospect of day by day practice. Moreover, regional society related and political issues may play a role, if it comes to implementation.

Food animals in their chain:

Surveillance systems for food animals are complex, requiring insight into the chains, needing appropriate verification/ control measures also with intervention plans in case of need. Verification techniques should focus on the whole chain reflecting the history of the animals/ herds in their site of origin, followed by observation of individual animals/ herds at the abattoir (as ante mortem and post mortem inspection). Techniques for each of these targets come from various disciplines (pure observation, chemistry, microbiology, histology, sensory (clinical inspection/ necropsy) or data processing (based on Information Technology) In addition, personal skills, infrastructure, target adjusted administrative background are needed. This paper offers examples for such structures.

KEYWORDS: Food Chains, Globalisation, Meat Inspection, Food Safety

BIOGRAPHY

Prof. Dr. Reinhard Fries
University Professor, Institute of Meat Hygiene and Technology,
Faculty of Veterinary Medicine, Freie Universität Berlin, Germany and
Head of FAO Reference Center for Veterinary Public Health

Prof. Dr. Reinhard Fries is Head of the Institute of Meat Hygiene and Technology, Freie Univarsität Berlin, Germany. Recently he is also an Executive Director of the FAO Reference Centre for Veterinary Public Health at the Faculty of Veterinary Medicine of Freie Universität Berlin, Germany which is regarded as centre of excellence in providing technical expertise, diagnostic services, laboratory and field training, in coordinating research and developmental studies, and in contributing to FAO projects. His academic interest is focused on poultry meat hygiene, meat inspection and related surveillance systems, quality control measures, food microbiology, veterinary public health, animal welfare (food animals), risk assessment application, hygiene in food animals. Prof. Dr. Fries graduated in Veterinary Medicine (DVM), Veterinary School of Hannover, Germany.

Long-term Monitoring of ESBL-producing *E. coli* in Broiler and Pig Fattening Farms and in their Vicinity

Uwe Roesler, Christina von Salviati, Henriette Laube, Anika Friese
Institute for Animal Hygiene and Environmental Health, Centre for Infection Medicine, Department of Veterinary Medicine, Freie Universität Berlin

SUMMARY

In this ongoing project long-term investigations concerning the occurrence of ESBL-/AmpC-producing Enterobacteriaceae are carried out inside and in the vicinity of seven pig and broiler fattening farms, each. Therefore, one barn of each farm is investigated three times within one fattening period (at the beginning, in the middle and in the end). The results show a high occurrence of ESBL-/AmpC-producing Enterobacteriaceae, especially *E. coli*, inside the barns and also findings in the surrounding of the farms. Even the most one-day-old chicken were tested positive for these resistant bacteria which leads to the question of the origin of these resistances and of vertical transfer, respectively. Concerning pig farms the detection level of ESBL-/AmpC-producing resistant *E. coli* was also very high. The investigated pig farms differ in their detection frequency of the ESBL-/AmpC-producing *E. coli*, some farms had a continuous low frequency in the animal samples others a very high level over all sampling times. Sampled broiler farms showed all high levels. Also the animals´ direct environment inside the pig and broiler barns was tested positive for ESBL-/AmpC-producing Enterobacteriaceae which could be one important transmission way of the bacteria within a flock. The frequent findings of these resistant microorganisms in dust samples originating from broiler farms in contrast to them from pig farms is striking. In air samples, however, ESBL-/AmpC-producing resistant *E. coli* were found rarely. In parallel, surfaces of the ground around the barns as well as exhaust air samples were taken. ESBL-/AmpC-producing resistant *E. coli* were infrequently detected on the ground in different distances from the barn, furthest in 500m. Only very few samples of exhaust air were tested positive. In principle, emissions of ESBL-/AmpC-producing Enterobacteriaceae via the spread of dust but more probably via the faecal way (fertilization of fields) seem likely.

BIOGRAPHY

Prof. Dr. Uwe Roesler
University Professor, Institute of Animal and Environmental Health,
Faculty of Veterinary Medicine, Freie Universität Berlin, Germany

Uwe Roesler graduated in Veterinary Medicine (University of Leipzig) in 1997. He obtained his PhD in Veterinary Medicine in 2001. He has been certified specialist in Animal Hygiene since 2001, certified specialist in Microbiology since 2003, diplomated of the European College of Porcine health management (ECPHM) since 2007, and certified specialist in Epidemiology since 2008. He was an assistant professor (University of Leipzig) in 2007-2008. Now, he is a professor for Animal Hygiene and Infectiology (Freie Universität Berlin), Head of the Institute of Animal Hygiene and Environmental Health.

EcoHealth/One Health – A Research Paradigm Shift Leading to Better Policies

Prof. Dr. Dirk Pfeiffer

University Professor, Department of Production and Population Health, Veterinary Epidemiology, Economics and Public Health, The Royal Veterinary Collage, University of London, United Kingdom

SUMMARY

Over the last two decades the world has become much more closely connected, not just electronically but also physically through vastly increased frequency and spatial coverage of human travel and trade in livestock-related and other products. These developments have contributed to the emergence several zoonotic disease threats of global significance since the beginning of the 21st century, including SARS, influenza A subtypes H5N1 and H1N1, as well as antimicrobial resistant pathogens such as MRSA. Furthermore, the increasing complexity of food supply chains represents a significant food safety challenge, as demonstrated during the melamine tainted milk scandal in China in 2008, EHEC 2011 epidemic in Germany and the horsemeat scandal in Europe in 2013. The global risk landscape has changed enormously and requires new approaches to risk governance. One aspect of this will have to be an integrated approach to research and policy development that takes the complexity of the underlying system into account. Particular relevance has to be given to researching the role of social drivers of emergence, in addition to biological and environmental factors. The recent trend towards a wide recognition of the relevance of Ecohealth and One Health approaches amongst policy makers provides a convenient platform for institutions to develop or strengthen their capacity in integrated research and policy development. But this represents a significant challenge due to the long-established disciplinary focus of academic research and the uni-sectoral thinking amongst most policy developing institutions. Policy makers on the other hand are more directly affected by ineffective policies, and therefore are more likely to recognize the importance of interdisciplinary and –sectoral approaches. The multitude of scientific disciplines present in academic institutions should be seen as an ideal opportunity for enhancing integrated research capacity, although the incentive for this to occur probably has to come from funding agencies and other external stakeholders.

BIOGRAPHY

Prof. Dr. Dirk Pfeiffer
University Professor, Department of Production and Population Health,
Veterinary Epidemiology, Economics and Public Health, The Royal Veterinary Collage,
University of London, United Kingdom

Dirk Pfeiffer graduated in Veterinary Medicine in Germany in 1984. He obtained his PhD in Veterinary Epidemiology from Massey University, Palmerston North, New Zealand in 1994, and worked as an academic in New Zealand for 9 years. He has been holding the Chair in Veterinary Epidemiology at the Royal Veterinary College (RVC) since 1999. Dirk has been involved in epidemiological research since 1985 and worked on animal health issues in developing as well as developed countries. He is the Head of both the Veterinary Epidemiology, Economics & Public Health Group and the newly designated FAO Reference Centre for Veterinary Epidemiology at the RVC. He teaches epidemiology at undergraduate and postgraduate levels and has designed and taught international training courses in veterinary epidemiology, risk analysis and spatial analysis in Europe, North America, Australasia and Africa. Dirk provides scientific expertise to various national and international organizations.

Knowledge, Risk Perception, Behavior and Diarrheal Diseases Burden among People in High and Low Diarrheal Incidence Areas of Northern Thailand

Akeau Unahalekhaka[1] Tongkorn Meeyam[2] Duangporn Pichpol[2]
[1] *Faculty of Nursing, Chiang Mai University, Thailand*
[2] *Faculty of Veterinary Medicine, Chiang Mai University, Thailand*

SUMMARY

Chiang Mai is the tourist spot for northern region and has high diarrhea incidence in Thailand. This studied aimed to determine food consumption knowledge, practices and behavior in people in the community in high and those in low diarrheal incidence areas. The approach for this observation based on transdisciplinary and ecosystem which combines quantitative and qualitative methods. Two villages with high diarrheal incidence and 2 villages with low incidence have been defined on the basis of national surveillance database and local medical office. Household food consumption habits between high and low incidence area showed statistical significantly different (p≤0.05) including sources of purchase raw food, eating outside, consider accredited label, eating hot food, washing hand before eating and after using toilet, wash fresh fruits and vegetable with enough clean water before eating, wash food utensils (spoons, plates, dishes) with dishwashing liquid, keep food left out the refrigerator and pest protect. Moreover, environmental practices were found statistical significantly (p≤0.05) as dispose of food scraps and all types of refuse by placing in waste containers with lids and burning refuse or rubbish. Perceptions of the risks regarding severity of diarrhea including eating meat from sick animals and individual eating practices presented different answers in low and high incidence area (p≤0.05). Contaminated food with pathogenic organism and flies, mice, cockroaches being vector of severe diarrhea, separating utensils for raw and cooked food, taking anti-diarrheal medication in diarrhea and proper disposing of refuse were remarked in knowledge of foodborne disease and prevention measures.

BIOGRAPHY

Assoc. Prof. Dr. Akeau Unahalekhaka RN, Ph.D.(Epidemiology)
Faculty of Nursing, Chiang Mai University

Education: B.Sc. (Nursing), M.S. and Ph.D. in Epidemiology Certificate in Surveillance and Applied Epidemiology for HIV and AIDS. Centers for Disease Control and Prevention (CDC), USA. Certificate in Statistical, Epidemiological and Operational Methods Applied in Medicine and Public Health. University of Brussels, Belgium. Diploma Thai Board of Nursing Care of Infectious Disease Patient and Infection Control of Thailand Nursing and Midwifery Council.

Past Position: Disease Control Officer, Chief of Planning & Evaluation Section, Division of Epidemiology, Ministry of Public Health, Bangkok, Thailand. Executive Board member of the International Federation of Infection Control (IFIC) and Asia Pacific regional coordinator. Council member of the Asia Pacific Society of Infection Control (APSIC).

Present Position: Associate Professor. Faculty of Nursing, Chiang Mai University, Thailand Consultant and supervisor in Infection Control among hospitals in Thailand. Consultant of Society of Central Sterile Supply Department (CSSD) of Thailand.

ORAL PRESENTATIONS

Smallholder Livelihoods and Animal Health in the Greater Mekong Sub-Region: Lessons from H5N1

Samuel Heft-Neal[1*] David Roland-Holst[1*] Joachim Otte[2]
[1] University of California, Berkeley
[2] UN/FAO
*Corresponding author; Email: sheftneal@berkeley.edu

ABSTRACT Since its emergence, H5N1 HPAI has attracted considerable public and media attention because the viruses involved have been shown to be capable of producing fatal disease in humans. While the virus may eventually mutate into a strain capable of sustained human-to-human transmission, the greatest impact to date has been the harm inflicted on the highly diverse poultry industries in some affected countries. HPAI control measures have so far focused on implementing prevention and eradication measures in poultry populations, with more than 175 million birds culled in Southeast Asia alone. In response to this, FAO and UKAID conducted a three-year research project assessing the efficacy of risk reduction measures and their effects on the livelihoods of smallholder farmers and their families. The specific purpose of the project was to aid decision makers in developing evidence-based HPAI control measures at both national and international levels that will be effective in terms of disease risk management, cost control, and social outcomes, protecting and enhancing the livelihoods of the region's rural poor majorities. In this synthesis, we present the many lessons of a four-country case study, with particular insights regarding smallholder producers in developing countries, who are and will remain the majority of livestock producers for the foreseeable future.

KEYWORDS: *H5N1, HPAI, poultry, risk reduction, smallholders*

INTRODUCTION

H5N1 HPAI has attracted considerable public and media attention because the viruses involved have been shown to be capable of producing fatal disease in humans. It's greatest impact to date, particularly the in the Greater Mekong Sub-Region (GMS) has been the harm inflicted on the highly diverse poultry industries in affected countries. HPAI control measures have so far focused on implementing prevention and eradication measures in poultry populations, with more than 175 million birds culled in Southeast Asia alone.

Poultry production in the GMS is heterogeneous, with the use of different species, different production and marketing systems and the provision of a range of products and services. Typically, poultry are an integral feature of smallholder agriculture, where the majority of households keep a small flock of 'indigenous', dual-purpose birds to meet household consumption needs, social obligations and minor cash expenses, the latter by sales through informal, live bird marketing channels. This traditional, extensive poultry production system is virtually ubiquitous and comprises by far the majority of poultry producers. Traditional, low-volume

poultry supply chains support livelihoods across extended networks of low-income people through production, distribution, processing, and marketing.

The specific purpose of the project was to aid decision makers in developing evidence-based HPAI control measures at both national and international levels that will be effective in terms of disease risk management, cost control, and social outcomes, protecting and enhancing the livelihoods of the region's rural poor majorities. In this short paper, we present an overview of lessons of a four-country case study, with particular insights regarding smallholder producers in developing countries, who are and will remain the majority of livestock producers for the foreseeable future.

MATERIALS AND METHODS

Events associated with HPAI outbreaks, including control measures adopted in response to them, appear to have seriously upset the gradual transition of the GMS poultry sectors. To better understand these impacts, and their implications for the viability of smallholder farmers, this study conducted detailed surveys in and around major poultry producing regions in Cambodia, Laos PDR, Thailand, and Viet Nam.

In all four countries, surveys were conducted with market actors including farmers, aggregators (i.e., traders), market vendors, and consumers. In total, between 1,000 and 3,000 surveys were conducted in each country. Actors along each stage of the supply change were asked about their roles within the supply chain, experiences with disease outbreaks, attitudes toward disease risk, and perception of overall risk in the poultry sector. In addition, information was collected about smallholder experiences with the public control measures that were implemented in response to outbreaks. Within each country, study sites were selected based on poultry production characteristics and past HPAI outbreaks. Respondents were then randomly selected within each site in order to be representative of rural poultry farmers in the areas (Heft-Neal et al 2009a).

RESULTS

Our detailed investigations of the smallholder poultry supply chain in Thailand, based on interviews with consumers, farmers, ex-farmers, farmer networks, traders, and vendors, suggests that recent changes in market conditions, as an indirect result of the HPAI outbreaks, are making it very difficult for small-scale poultry farmers to sustain their enterprises in Thailand and, in some cases, Viet Nam. At the same time, the small-scale poultry sector in Laos PDR and Cambodia have remained largely unchanged.

Commonalities across study countries that were identified from the surveys include the role of poultry in livelihoods and smallholder incentives to participate in disease control. Poultry was found to play an important role in the livelihoods of most rural households. Moreover, while most backyard farms consist of only a small number of chickens, these livestock serve an important function within the household. Poultry was found to account for 0-35%, but typically <10%, of total cash income. Consequently, even though the majority of (poor) households can withstand one-time losses of their poultry, reduction / foregoing of poultry income was found to negatively affect within-household bargaining power and expenditure allocation of women, which is particularly targeted at safeguarding the welfare of children.

Other features of the study areas were found to be quite variable. Reported strategies for coping with HPAI (but more so with HPAI control measures) of the poor are very diverse and included foregoing consumption, taking children out of school, rural – urban migration in search of alternative income opportunities, etc. In Thailand, our results suggest that recent changes in market conditions, as an indirect result of the HPAI outbreaks, are making it very difficult for small-scale poultry farmers to sustain their rural enterprises. Despite the absence of large outbreaks since mid 2004, we observed significant movements out of the native chicken sector during 2006 and 2007. Households who grew chicken in the past continue to do so for own consumption, but

they presently see sharply diminished prospects of a livelihood from this form of livestock. In contrast, conditions for smallholder poultry production were found to not have significantly changed in Cambodia or Laos PDR while the situation in Viet Nam was found to be somewhat intermediate with 'erratic' application and lifting of poultry production bans and plans for livestock production zones.

DISCUSSION

One of the main outcomes of the study was significantly better understanding of how existing institutions and stakeholders operate and interact within supply chains. It became evident that trust, reliability, and market information are main components of these relationships. Moreover, while some commonalties emerged, there were also many distinctive features of local poultry supply chains in each country.

Integrated industrial poultry production is well established in Thailand and emerging in other Mekong countries, targeting both urban and export markets. Simultaneously, however, intensive, industrial poultry production systems have been established, particularly in Thailand. The traditional extensive and the industrial poultry production systems are extremes, between which 'hybrid'/ 'intermediate', commercial/ market-oriented systems exist, combining characteristics of the other two (e.g. partial scavenging with feed supplementation, indigenous birds crossed with industrial poultry lines, thereby relying on 'formal' input supply systems), operating at intermediate scales (hundreds to several thousands of birds), and mostly relying on 'traditional', informal live bird marketing networks. Each production model has advantages and disadvantages and none is likely to disappear completely. In Thailand, large-scale industrial poultry production is one of the economy's most important sources of animal-derived food, employment, and income. In Cambodia, the 'formal', industrial poultry sector occupies a minor share in national poultry production, while the

situation in Viet Nam is intermediate between that of Thailand and Cambodia.

Although the market share of smallholder poultry production is diminishing in many regions as the industrial poultry sector expands, market-oriented smallholder producers still constitute the vast majority of 'commercial' poultry production units. In the GMS, as elsewhere, their market interactions are governed by verbal agreements and informal contracts - smallholders and small enterprise downstream intermediaries are deeply embedded in networks of customary trading and mutual insurance. Trust, reliability, credit, conflict resolution, and contract enforcement are main components of these relationships.

Features of these smallholder production systems should be taken into account when developing policy responses to disease outbreaks. Farmers are used to recurring large-scale poultry losses from a variety of poultry diseases and can cope with a 'once-in-a-while' loss of their poultry stock by moderately increasing other agricultural activities. The majority of Viet Nam's and Cambodia's poor smallholder poultry keepers live in the densely populated lowlands, where market transactions and movement of goods, livestock and people are frequent.

Policies that disrupt livelihoods may drive production and trade underground and thereby unintentionally increase disease risk. On the other hand, allowing the regional poultry trade, in its current form, poses risks to public health and large-scale producers, in addition to the risks posed to small-holders' poultry and their own health. In all GMS countries, HPAI responses were, at least initially hampered by conflict between the Ministry of Health and the Ministry responsible for agriculture, and within the latter, by rivalries between departments, e.g. production and animal health.

In Thailand poultry exports drive HPAI responses. The major export-oriented producer companies are represented in their interactions with government officials and the media by a handful of highly organized and sophisticated lobbying groups. Many

decisions taken by Thai officials reflect direct input from and / or collaboration with the industrial lobby whose main concern was to maintain / regain their access to global export markets. To their credit, the industrial producers contributed financially to a scheme to boost compensation to smallholder farmers who surrendered their birds.

In Viet Nam, fragmentation of authority within the structure of government from central to local levels constrains HPAI responses. Central policies become a channel to distribute patronage and major discrepancies exist between central and local policies, and between policy intents and policy results. Implementation of policies is extremely contentious, with competition for resources intersecting with competition for power.

In Cambodia, poor governance and institutional failures are exacerbated by strong patronage politics and power monopolies. Corruption, quarrels, rivalries and competition between political parties, ministries and departments hamper collaboration and goal achievement. The private sector is not a driving force in HPAI control.

CONCLUSION

Given the structure of current market incentives, smallholder poultry keepers are unlikely to adopt compulsory bio-security measures. Diseases are part and parcel of their everyday experience and local responses are determined by local cultural rather than by imposed technical rationales. Therefore, any attempt to formalize markets without maintaining low transactions costs will displace low income participants across low income food supply chains, undermining food security and livelihoods for countries with low income majorities. Mitigation of collateral impacts through supporting coping mechanisms, however, is likely to enhance social effectiveness of public and private HPAI risk management programmes. There is a direct link between the perceived value of poultry and the optimum disease management approach. Higher valuation of live poultry will increase the care taken,

possibly enhancing monitoring efforts and thereby reducing the culling radius. Enhancing the value of poultry, via improved marketing and safety, would ultimately result in less drastic HPAI control policies.

Development of incentive-compatible policies critically depends on information technologies. The time lag between infection and detection, both at the bird and flock level, will affect policy design and the impact of these policies. When, in an ideal situation, detection is low-cost and immediate, one can introduce incentives like penalties for not reporting sick animals and having them culled. A penalty that is equal to the 'social cost' of not culling is 'optimal', and is superior to a subsidy for culling (compensation for sick birds) because the subsidy will result in over-production and under-investment in prevention. Also, when information is imperfect, 'ring' culling is a crucial disease control measure. Earlier (and more accurate) information will reduce the optimum radius of culling and thereby spare resources and livelihoods.

The need for improved disease surveillance is global, willingness to pay at each location may be small, but gains may be substantial. Based on a simple statistical value of life calculation, we estimate that the gain from reduced pandemic risk is in the billions of dollars, annually. The private sector is unlikely to invest optimally in development of improved surveillance and risk reduction measures. Therefore, development of disease surveillance technologies has a global public good element, and their development should be supported by public sources.

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Caring Harmonization in Life among Animal, People and Environment through Monitoring of Population and Analysis Vegetation of *Macaca fascicularis* in Semarang, Central of Java, Indonesia

Pudji Astuti[1,2,*] Arif Setyawan[2] Asmarani Kusumawati[1,2] Luthfiralda Sjahfirdi[3] Hera Maheshwari[4] FX Wagiman[2]

[1] *Faculty of Veterinary Medicine, Gadjah Mada University, Indonesia*
[2] *Center for Management of Biological Resources Study, Gadjah Mada University*
[3] *Faculty of Mathematics and Sciences, University of Indonesia, Depok, Indonesia*
[4] *Faculty of Veterinary Medicine, Bogor Agricultural University, Bogor Indonesia*
Corresponding author; Email: Pastuti2@yahoo.com

ABSTRACT Most of habitat *Macaca fascicularis* in Kreo cave has been converted into dam for the benefit of human such as preventing flooding. Better monitoring of population of *Macaca fascicularis* is needed, especially for inhibition in escaping animals to the local communities and where such animals may become important animal reservoirs maintaining disease transmission in the face of public health control. Research has been conducted on *Macaca fascicularis* for 1 month/year during 3 years, started in 2010 until now. Population was counted by Track and count method; distribution and movement of Macaca was obtained by following them daily for 7 days then analyzed using google earth software with The extension *KML* tools project. The position of sleeping and food area has been recorded by Global Positioning System (GPS) and mapped. Besides of population and daily range, data of behavior, and analysis of vegetation (it was not presented in this conference) were obtained. The result showed in 2010, there were a total of 201 individu which were contains of 134 animals from Kreo (female 21%, male 10% juvenile 45% and infant was 15%) ; and 67 animals from Parkiran (female 25%, male 18%, juvenile 42% and infant was 15%). In 2013, number of group became 3 since Kreo group were divided into 2, so that all of composition are as followed: Kreo group I: 110 animals and Kreo Group II: 34animals. Individu of other group namely Parkiran are 94 individu, (62% adult, 31,9% juvenile and 2,1% infants). Based on habitat, they loss more than 75% as previous area. Twelves of 17 feeding tree has been disappeared. Daily range of all groups were fluctuated, and rate of density is 11.48 Individual/Ha in 2010 to 41.69 individual/Ha in 2013. It has been concluded, number of monkey is increase during 3 years, and part individual have escaped and formed a new group since of competition for feed, tree of sheltering and daily range. Replanting of trees should be implemented on a large scale to replace the lost trees as feed and sleeping.

KEYWORDS: *Macaca fascicularis, population, feeding tree, habitat*

INTRODUCTION

Zoonotic is a number of infectious diseases, including viruses, bacteria, and parasites, can be transmitted from animals to people through a variety of infection routes, including animal bites, vectors (i.e., insects), and animal-to-human contact (i.e., inhalation of respiratory droplets or skin-to-skin contact). Non-human primates can carry a variety of zoonotic diseases such as influenza virus, measles, pox virus Ebola, Tuberculosis, (OIE , 2010). Emphasis on non-human primate which located at the tourism site like Kreo cave, care should be taken by managing population, of *Macaca fascicularis* to prevent potential exposure to zoonotic pathogens since more than 75% of their habitat has been dissappeared because of dam construction so that density is getting increase sharply. Even a few, monkey has started expand to the local community, it can become a serious local pest and harmonization in life among animal, people and environment will be disappeared. As our responsibilities to the conservation of *Macaca fascicularis*, monitoring study has been done since year 2010 when the Jatibarang dam was built up until be finished in 2014. By knowing the number of population its will be predicted rate of reproduction and also density of population in Kreo cave. Thus, the next step and recommendation will be given clearly. The aims of this study area; to count population; b. to assess their ecological aspect as impact of Jatibarang-dam development; c. to find out recent population and distribution of Long tailed macaque , and d. to asses habitat of Long tailed macaque in the Kreo-Cave tourism-site.

MATERIALS AND METHODS

Macaca fascicularis that lives in Kreo cave was an object of this research. Generally, they look for food at surrounding area of the cave, but the Local Tourism Agency supplied for food such as corn and casava once per day.

Study of Population

Based on the area condition of Kreo cave such as small and no flat with certain area for moving, track and count method (NRC, 1981; Steiner, 1999) was used to estimate the population of *Macaca fascicularis*. In this method, observer followed the macaque groups and counting their numbers in their feeding sites and sleeping sites.

The First step, information of group size, estimation of number population, sleeping tree (sleeping area) and food sites (food area) of *Macaca fascicularis* was obtained by interviewing local communities. The position of sleeping and food area were recorded by Global Positioning System (GPS). Estimation of population (Structure of population) was done during ten days. The highest data on population density was estimated as density and excessive population of Long-tailed macaque. The habitat value was obtained by making spotted pattern 20 x 20 meter^2 in specific sites which be used for activities such as home range, sleeping and feeding area. Tree vegetation with diameter 8 cm or more was recorded and measured by length of the chest.

RESULTS

During 10 days survey in April 1st - April 10th, 2010, it has identified two groups of Long tailed macaque in cave which was called as "Kreo" and "Parkiran" group. Kreo group has 134 individu and Parkiran 67 animals, so that total population was 201 animals. For Kreo group, juvenile was dominant, followed by adult female, infant, and adult male, whereas Parkiran which was dominated by juvenile, adult male, adult female and infant (see table 1). Three years later namely 2013, number of group was getting increase into 3 group such as Kreo I (110 individu), Kreo II (34 individu) and Parkiran (94 individu) with the total individu is 238 animals. Composistion of animals was dominated by adult (60.3%), juvenile (34.03%) and infant (5.04%).

The area occupied by these monkeys were 9.79 Ha (according to Tourism agency map), thus population density of long tailed macaque 9.8 - 20.5 individual/ha. In 2013, rate of density is 41.69 individual/Ha. Their habitat has lost more than 75%. Daily range from 3 groups are fluctuated, and 12 from 17 of feeding tree were dissapear.

Group Composition of Kreo

Group Composition of Parkiran
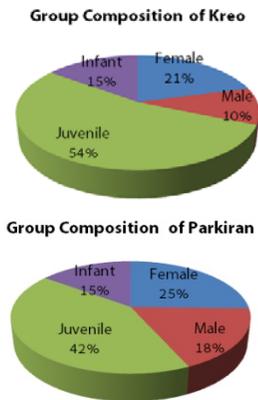
Figure 1.1: Composition of monkeyin 2010, it was only 2 group (Kreo and Parkiran).

Composition of Kreo group

Composition of Kreo-1

Composition of Parkiran
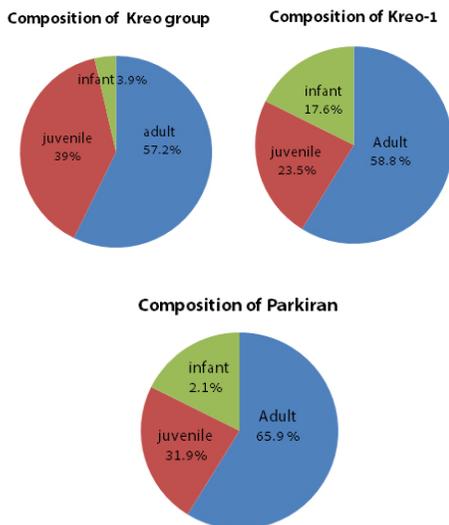
Figure 1.2: Composition of monkey in 2013, Kreo group was devided into 2 group so that number of group become 3.

DISCUSSION

1. Population of Macaca fascicularis

Monitoring of long tailed macaca is very important since primate has contribution of zoonotic deasease such as influenza virus, measles, pox virus Ebola, Tuberculosis, (OIE, 2010). Besides zoonotic, over population of Macaca could destroy the crop of local community so that they fail harvest. Local agencies are charged with removal and translocation of problem monkeys, including trapping of rhesus macaques to be used for research within India (Malik & Johnson, 1991; Malik, 1992; Southwick & Siddiqi, 1994. (Cit.

Crocket et al., 1996). In Barbados, humane trapping was introduced. In this research, many activities to be done to maintain population and daily range in order to obtain harmonization in life without removal and translocation, since Macaca is not a pest regarding before. Similar research such as monitoring population in other tourism site also be done, and the community perception agreed to develop the ecotourism forest area because if can increase their earning. The development of ecotourism can be done in manner of the development of potential object, local community empowerment, institutional, intensive marketing and promotion, and the primate conservation for ecotourism (Muhibbudin, 2005).

Table 1: Dynamic of Daily range of Kreo group start from 2010-2013.

(KG)	Daily Length Movement of Kreo			
Day	Apr-10	Mar-11	Nov- 12	Feb-13
1	688	848	975	922
2	511	828	987	691
3	1599	984	998	880
4	954	768	931	1111
Average	938	857	972,75	901

In this research, increasing number of group from 2 become 3 groups are caused by limited of feeding, habitat and sleeping tree. Female long-tailed monkeys usually would be with a group where she was born, and the males will usually leave the group in which he was born and became a prolific males in other groups (de Jong et al, 1994). It is very likely to occur with Kreo, where the number of individuals has decreased overall, it is possible to form a new group and have their own home ranges. Fürtbauer *et al.,* (2009) reported that activities can affect status of reproduction. Besides to form of new group, there are many possibilities of decreasing individual such as predator, death, escape to another area and disease. According to public information about (Gino, personal communication 2012), there is a new group of monkeys who are not from the two groups that have been identified (Kreo and Parkiran) around the dam is being invented, unfortunately, it cannot be detected when the

observation was running because of bad climate such as raining all of the day.

Table 2: Dynamic of Daily range of Parkiran group start from 2010-2013.

(KG)	Daily Length Movement of Kreo			
Day	Apr-10	Mar-11	Nov- 12	Feb-13
1	288	769	427	290
2	203	724	495	242
3	522	710	717	289
4	307	732	713	324
Average	330	733.75	588	286.25

Table 3: Daily range of new group (KG-1).

KG-1	Daily range of the new group	
Day	Nov- 12	Feb-13
1	872	592
2	1315	494
3	757	668
4	837	448
Average	945.25	550.5

2. Daily Range of Macaca fascicularis

In this monitoring, daily range of Macaca both for Parkiran and Kreo group was similar as last monitoring eventhough a little bit longer. From time to time, in the dry season, there was a tendency of group of Macaca has more longer and wider daily range as explained as below:

Daily Range of Parkiran Group. Comparison to the previous monitoring in February which was rainy season, stated daily range was longer. The last of June until early of July was entering to dry season, it meant too was getting difficult to obtain some water, trees of feeding and sheltering has begun reduced, so that the monkeys will find places that are more convenient. Fortunately, feed the monkey was overflow so animals do not expand to the local community to steal crops. This fact is opposite with chimpanzee in savana as reported by Pruetz and Bertolani (2009) combined with data on temperature in the various habitats within the savanna mosaic, results show that Fongoli chimpanzees minimize energy expenditure during the hottest months and at the hottest time of day by resting more and traveling less, in addition to selectively using small patches of closed-canopy habitats, such as gallery forest. They move significantly more during early hours of the hot, dry season specifically and range in smaller parties at this time compared to during the wet season. The stresses associated with a savanna-mosaic environment and chimpanzees' behavioral adjustments to them have important implications for understanding early hominin behavior in similar environments. Berman et (2007) reported infant mortality is good indicator of the impact of tourism. In this research, none infant mortality was found.

Daily range of the new group indicated that narrower than before since in rainy season there are many kind of feeding, so that animals do not look for feeding in the long area. This condition is opposite as the dry season when feeding was very difficult to be obtained.

CONCLUSION

Based on results and discussions, it would be concluded that:

1. Number population of *Macaca fascicularis*, is getting increase during the periods of 3 years.

2. Number of group of Macaca fascicularis is getting increase from 2 become 3, since competition of feed, daily range and lack of habitat.

3. Monitoring of *Macaca fascicularis* must be done to prevent animals escaping to the local community.

4. The growing plants must concern of characteristics function as feeding, covering or aesthetics. It would be better to choose plants which resistent on weather

5. Although most of habitat has been disappeared, population is getting increase, harmonization among people, animals and enviromental in Kreo Cave still stable.

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Telephonic Trading Systems for Smallholder Livestock: Opportunities and Challenges for Technology Adoption

Drew Behnke[1*] David Roland-Holst[2] Joachim Otte[3]

[1] University of California, Santa Barbara

[2] University of California, Berkeley

[3] UN/FAO

*Corresponding author; Email: dbenke@umail.ucsb.edu

ABSTRACT The emergence of HPAI in the Greater Mekong Sub-region sharply raised awareness of the need for decentralized systems of animal disease risk management. In an innovative effort to promote this, we worked with local poultry producers in Northern Thailand to introduce an SMS-based trading system for chickens. This telephonic auction trading system, the first of its kind, had several purposes, including facilitation of smallholder market access and livelihoods improvement. In addition, however, it acted as a de facto supply chain certification and traceability system and, with our sponsored delivery program, a voluntary animal health surveillance system. During the period of this trading pilot, every chicken passing from individual farms to urban vendors had its blood sampled and origin-destination registered. The results of our experience with this innovative system have implications for voluntary, sustainable, and self-financed animal disease risk management. This paper distils the lessons from our pilot project and lays out the next phase of this research, with special reference to the challenges of smallholder technology adoption.

KEYWORDS: *Information and Communication Technology, Mobile Phones, Asymmetric Information, Smallholder Poultry Production, Producer Welfare*

INTRODUCTION

The last three decades have witnessed dramatic transition of the Thai poultry sector, with emergence of large agrofood conglomerates that now produce 75% of all poultry, serving large urban markets and the world's second largest chicken export industry. Despite this, smallholder backyard poultry remain ubiquitous across Thailand's countryside, where they offer rural low-income households a complete array of financial and other services. Furthermore, households depend on the supplemental income generated from chicken rearing and households that have exited the market still often raise chickens for household use.[1]

In large part due to the informal nature of the indigenous poultry supply chain the market suffers from extreme inefficiency. This pilot project used simple mobile phone technology (SMS text messaging and interactive voice response menus) to connect rural farmers with market vendors in the surrounding areas of Chiang Mai. The project attempted to provide a low cost method by which to greatly improve market efficiency, increase market access for small-scale chicken farmers, reduce price distortions, and provide incentives for farmers to provide high quality chicken meat.

MATERIALS AND METHODS

The system that was developed, referred to as *eBird*, enabled small-scale native chicken

farmers and wet market vendors to easily communicate with simple mobile phone SMS text messaging. Participants also had the option to use a Thai language interactive voice response system if participants were unable to send SMS messages. The goal of the project was increase market access and participation for rural smallholder poultry farmers.

If a participating farmer had a group of chickens, a bundle, that he wished to sell he was able to open a proposal. He could send a text message with relevant information (number of chickens, weight, chicken type, etc.) to our system phone number, or alternatively he could call our e*Bird Hotline* where he was prompted to enter the information by the interactive voice response system. Once the proposal was entered, a text message was sent to all participating vendors in the farmer's area. A vendor that wished to purchase the available bundle then could respond to the SMS message with the transaction ID number to accept the proposal. This action closed the proposal and if other participants attempted to accept this proposal they were notified that it is no longer available. If proposals were not accepted within two days of opening the proposal then expired. Following closure of a proposal, a member of our team would visit a farmer's residence. The team member would pay for the bundle and deliver the live chickens to the vendor involved in the transaction at which time the vendor would pay our researcher the price of the bundle. The vendor would then house the live birds until the time of slaughter and transport to market.

In order to achieve good communication between researchers and potential participants a team of ten researchers was recruited from Chiang Mai University. Initial surveys were designed and conducted to gather information that would be important in tailoring the system to meet the particular needs of local poultry market actors. Another objective of preliminary surveys was to identify areas where clusters of potential candidates existed. We designed the functionality of the pilot to attempt to improve efficiency and market access while minimally diverging from current practices.

Once potential participant clusters were identified our survey team then proceeded to register and train the farmers and vendors in the area. All relevant information is gathered from the participants (name, mobile phone number, address and/or detailed location, etc.) and participants were issued identification and PIN numbers that were used when communicating with the system.

In order to train the participating farmers and vendors our research team demonstrated use of the system and assisted participants in entering initial transactions. Additionally researchers provided a packet of materials including an instruction sheet and a wallet size ID card. We also established a helpline that was available to users that experienced any problems with the system. Calling this number would reach a member of our team that could offer assistance.

As an incentive to encourage initial use and sustained participation, vaccinations were given to participating farmers. By issuing vaccines to our farmers we developed an important signaling strategy that we were invested in the farmer's production of indigenous chickens. Furthermore, when we vaccinated a farmer's flock we effectively claimed their birds as our own to be used in the *eBird* system. This is very important, as one of the biggest challenges was encouraging farmers to use our services instead of a traditional supply channel.

We vaccinated birds for Newcastle Disease, Infectious Bronchitis, and Fowl Pox. Vaccines were purchased from the Department of Livestock Development (DLD), and although they were inexpensive it appeared most farmers did not seek vaccinations on their own. This may be for a variety of reasons such as vaccinations are more expensive if purchased through a third party, farmers may not want to travel the distance and take the time to purchase from the DLD, they may not know where to get vaccinations, and they may not know how to properly administer vaccinations. Thus, our technical assistance in this field was especially important because it

provided a strong signal of investment in the farmer's flock for a low overall cost.

RESULTS

The *eBird* system became active on November 1, 2010. By April 9, after 160 days of active trading, 96 transactions were completed with a total of 373 native chickens being traded. At this point there were 328 farmers and 8 vendors actively participating in the pilot. During this period the average chicken traded on this system weighed approximately 1.17 kilos and sold for approximately THB 88.00 at an average of 75.43 THB/kilo. The information in Table 1 displays a brief summary of the transaction data.

Table1: Summary of farmer initiated transactions.

	Number of transactions	Number of Chickens	Weight of bundle (KG)	Price (THB)	THB/ KG
TOTALS	96	373	400.2	30,486	-
AVERAGES	-	3.89	4.17	317.6	75.43

Figure 1 below displays a chronology of system activity over the course of the first 23 weeks.
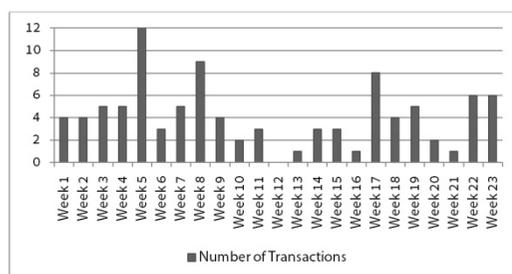
Figure 1: Number of Weekly Transactions (11/1/2010 – 4/9/2011).

As indicated by the figure above, fluctuations in the system were quite common with weekly transactions ranging from twelve to zero. Fluctuations in system activity are to be expected for a variety of reasons. First and most importantly, the nature of smallholder indigenous poultry production makes any sort of sustained stability in sales extremely difficult to attain. Birds take approximately 3 months, and often even longer, to reach a desirable market weight and as a result the majority of our participating farmers only sold once over the course of the project. Thus indigenous poultry production is characterized by long intervals between sales, which explains why sales often vary from week to week. Furthermore farmers often continue to sell birds through traditional channels, and thus it is by no means guaranteed that all farmers will utilize *eBird* in a three-month period. Generating stable sales on a weekly basis therefore has two requirements. First, the farmer database must be sufficiently large to draw birds of market weight on any given day. Secondly, time is needed to demonstrate that *eBird* is a real and viable alternative to traditional supply-chain channels.

Figure 1 also demonstrates the seasonal nature of poultry sales. Weekly sales depend on a variety of social factors as well such as the prevalence of holidays where birds are sold in large quantities. Before these holidays approach sales often slow down as farmers prepare their flocks for personal needs or increased marketing opportunities. This partially explains the dip in sales during weeks 20 and 21 as these dates precede Thai New Year, one of the largest festivals of the year.

DISCUSSION

Although the volume of use of the eBird platform was relatively low, the system was quite well received among both farmers and vendors in the area. We recognize that the adoption of new technology in a very low-tech sector is difficult and thus we expect that volume of use will only increase gradually. That being said, nearly 100 transactions were completed in a short period of time, indicating some farmer's willingness to utilize the new technology. Furthermore, we believe that with a sustained presence in the area, this system can meaningfully affect the efficiency of the native chicken market and in turn increase the incomes of the small-holder chicken farmer.

The concept of establishing a long-term sustained presence is vital to the success of the project for numerous reasons. If the technology will ever be truly adapted in a natural manner, farmers must gain experience

utilizing the technology. Unfortunately sales opportunities were limited as indigenous poultry production is a time-intensive operation and birds typically require 12 weeks to reach market weight. Therefore, a typical farmer may only sell birds 4 to 5 times over the course of the year, giving them limited opportunity to familiarize themselves with the technology. Given this, the first 160 days of system use discussed in this paper represent a very early stage in the development of the eBird system. It is only after a period of 12-18 months can the success of the system truly be gauged as it will give the participants enough opportunity to learn and utilize the system independently.

In theory, increased confidence in the eBird system as a viable means by which to sell chickens at any time and at high prices per KG, will encourage farmers to increase holdings. However in practice we did not see farmers in Chiang Mai increase their holdings as a result of eBird. This can partially be explained by the limited time the system was in operation in Chiang Mai. Additionally, and likely a more significant factor, is the relative wealth of farmers in the immediate areas surrounding Chiang Mai. These areas are relatively well off compared to more impoverished locations in Thailand, and thus farmers are generally not interested in expanding production of poultry as they have other more reliable and sizeable sources of income. Thus, the real success of the eBird system should be tested in an area characterized by subsistence agriculture where farmers may better respond to the incentive to increase capacity. Regions such as the Issan of northeast Thailand or Southern Laos would be ideal for this as the countryside is dominated by smallholder subsistence production.

In order to achieve sustained use of the system this service must provide real long-term benefits to participants. With native chicken demand exceeding supply the potential benefits to farmers that successfully increase chicken holdings are great. Increased farmer usership of the system and increased native chicken holdings would in turn provide vendors with increased supply chain reliability. Better information and greater farmer accountability also introduces incentives for farmers to provide high quality chickens. The market as a whole will then benefit from increased efficiency and quality. Quality improvements then may lead to increased consumer confidence in native chicken meat. If such benefits are realized long-term sustained use of the system is likely.

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Zoo Change Management toward One Health Leadership: The Role of Veterinary and Leadership Approach

Putratama Agus Lelana [1,*]

[1] *Faculty of Veterinary Medicine Bogor Agricultural University*
Corresponding author; Email: aguslelana@gmail.com

ABSTRACT Zoo vision and mission statement analysis was done in order to perform initiative of zoo change management toward one health leadership. The consideration of this study was based on the fact that wildlife animals have potential role as a resources and reservoir of zoonotic diseases. Preventing zoonosis as well as promoting one health should be a major task of zoo in the future. The result of analysis was in line with the current perspective that zoos were viewed more as an entertainment or wildlife refuge than as repositories for data pertinent to one health issue. To perform the initiative of change of zoos, we noted 7 (seven) keywords related to one health concept. These keywords were used as a toll to analyze the readiness of zoo in implementing one health concept based on their current vision and mission statement. Based on an analysis of 25 zoos selected from 800 zoos in the world, we noted that 40% is possible to implement one health concept through the keyword education. Other keywords yielded are as follow: communication 4%, clinical care as well as diseases surveillance and control 16%, diseases transmission as well as prevention and control diseases 24%, and media publication 0%. The result suggested that zoo change management toward one health leadership should be performed. In order to perform this effort, personal and institutional readiness should be prepared as perfect as possible. In line with this preparation, conditioning of organization dynamic due to staff attribute and climate for change should be done. In order to evaluate the progression of zoo change management, the stages of change should be analyzed. This analysis should be addressed to the exposure, adoption, exploration, and practical implementation that reflect organization's culture. In this concern, the leadership approach has an important role in enhancing organization's culture as well as accelerating the program improvement. The veterinary approach was needed to fit organization's culture with daily activities that related to the implementation of one health concept. In this concern, veterinary leadership could be developed as an effective approach to engage one health leadership.

KEYWORDS: *zoo change management, one health leadership, vision analysis, veterinary, zoonosis*

INTRODUCTION

Every country in the world tried to magnitude the performance of zoo functions in exhibiting their own wild life animal biodiversity as competitive and comparative advantage (1). This reason could play an important role in zoo development and management. The developing zoos in the real world could be noted as educational

exhibition resources, ex-situ conservation, and natural tourist destination (2). However, the potential function of zoos in applying "one health" was not clearly and explicitly sounded.

Wildlife animal was considered as a complex creation that never free from mutual-parasitical symbiosis with micro-organism that potentially play a role as a diseases agent (3). The host of diseases could be addressed as definitive host that characterized by clear clinical diseases symptoms as well as un-definitive host that did not have explanation on clinical diseases symptoms (4). In this concern, wildlife animals could play an important role as a diseases resources as well as diseases reservoir, including zoonosis (5).

Climate change, global warming, and its influence to ecosystem seemed related with the increasing evidence of new emerging diseases or re-emerging diseases (6). This situation was already predicted 30 years ago and develops uncertain future of human animal relationship (7). This threat should be faced by worldwide strategy on expanding interdisciplinary collaborations and communications in all aspects of health care for humans, animals and the environment (8). We call this concept as one health strategy that should be enriched by integrated skills, managements, powers and it influences that are called as one health leadership.

Based on these explanations, we concluded that management of developing zoo should be implemented in a comprehensive way (9); not only for educational exhibition resources, ex-situ conservation, and natural tourist destination, but also toward one health leadership. One health leadership for zoos should be about changing the zoo's way of thinking and management to reflect their value for data and knowledge transfers of environmental-human-wildlife animal interaction, including sentinels, vectors, and diseases agents.

To enhance this stage, change management (10) supported by visioning one health concern as well as leading project management in personal and organizational zoo, should be implemented (11). This paper was proposing the initiative of zoo change management (12) towards one health leadership. To establish this initiative, vision and mission analysis of zoos was done resulting as follows.

MATERIALS AND METHODS

Vision-mission analysis was done for developing rationale of zoo change management initiative toward one health leadership. This study was done on May 2013 in the Faculty of Veterinary Medicine, Bogor Agricultural University, Indonesia.

The study was done by searching the information of vision-mission of zoos through internet. Screening was applied in three stages. The first screening was to list zoos having a website. The second screening was to list zoos whose websites state vision and mission. The third screening was a part of vision and mission evaluation. In the process of evaluation, each statement of vision and mission was graded in three categories: (1) whether it has an explicit statement related to one health concept, (2) whether it has an implicit statement that could be related to one health concept, and (3) whether it has no statement that related to one health concept at all.

One health concept could be described as 1) joint educational efforts between human medical, veterinary medical schools, and schools of public health and the environment; 2) joint communication efforts in journals, at conferences, and via allied health networks; 3) joint efforts in clinical care through the assessment, treatment and prevention of cross-species disease transmission; 4) jointcross-species disease surveillance and control efforts in public health; 5) joint efforts in better understanding of cross-species disease transmission through comparative medicine and environmental research; 6) joint efforts in the development and evaluation of new diagnostic methods, medicines and vaccines for the prevention and control of diseases across species; and 7) joint efforts to inform and educate political leaders and the public sector through accurate media publications (13).

Based on the above descriptions, keywords related to one health concept are 1) education, 2) communication, 3) clinical care, 4) diseases surveillance and control, 5) diseases transmission, 6) prevention and control diseases, and 7) media publication.

To reduce subjectivity, the interpretation of vision and mission statement was done by three person. In the interpretation activity, defining the keyword of vision and mission should be done carefully. From this keyword analysis, grading was performed. The result was then considered as a basis to develop zoo change management towards one health leadership.

RESULTS

There were almost 800 zoos distributed around the world. However, not every zoo has its own website. In Indonesia for example, only 12 out of 21 zoos recorded in google.com have a website.

Table 1: The resume of vision and mission statement analysis of selected zoos to related keyword of one health concept.

No	Related Keyword of One Health Concept	Percen-tage
1	Education	40
2	Communication	4
3	Clinical care	16
4	Diseases surveillance and control	16
5	Diseases transmission	24
6	Prevention and control diseases	24
7	Media publication	0

Grading the vision and mission statement of zoos was not easy. None of Indonesia's zoos mentions clearly a statement related to one health concept. A search through Google with the keyword of "zoo vision and mission statement" resulted in 25 zoos with a resume as described in Table 1.

The vision analysis showed that 40% of the selected zoos explicitly states education in the vision, that could be used as a basis to implement the one health concept. This explicit statement doesn't guarantee whether the zoos have applied the one health concept, unless it is stated explicitly in the zoo's action plan or programs. However, this statement

could bridge the zoos to certain activities related to one health concept.

Analog to this explanation, only 4% of zoos stated their vision with a keyword of communication. The benefit of having an explicit statement could be used as rationale approach to enrich the communication that related to one health concept.

In order to animal concern, we noted 16% of zoos had vision statement that indirectly related to clinical care and diseases surveillance & control. In order to animal-human concern, we noted 24% of zoos had vision statement that indirectly related to diseases transmission and prevention & control diseases.

Based on these results, there were open opportunity for zoos to develop change management towards one health leadership. The first important step should be performed by zoos were developing the new vision and mission that open to the implementation of one health concept.

DISCUSSION

The One Health concept is a worldwide strategy for expanding interdisciplinary collaborations and communications in all aspects of health care for the human, the animals and the environment. The synergy achieved will advance the health care of the 21st century and beyond by accelerating biomedical research discoveries, enhancing public health efficacy, expeditiously expanding the scientific knowledge base, and improving medical education and clinical care. When properly implemented, it will help to protect and save untold millions of lives in our present and future generations (13).

The keyword degraded from zoo vision and mission statements could be a bridge to certain activities related to one health concept. This aspect could be performed as a moving strategy from implicit knowledge to shared explicit knowledge. This aspect could also drive a new way of zoo's thinking that is important for internalization of one health concept among internal and external stakeholders.

Even though the keyword of zoo vision and mission statement could be a bridge to the implementation of one health concept, it doesn't guarantee that one health concept has been applied. However, zoo change management could be used as a soft skill tool to enhance one health leadership. How change management was considered as an effort of preparing readiness of personal and organizational in implementing program improvement towards one health leadership (11). In line with this preparation, the conditioning of organizational dynamic due to staff attribute and climate for change should be anticipated (14). In order to evaluate the progression of zoo change management, the stages of change should be analyzed. This analysis should be addressed to the exposure, adoption, exploration, and practical implementation of one health concept (Fig.1).
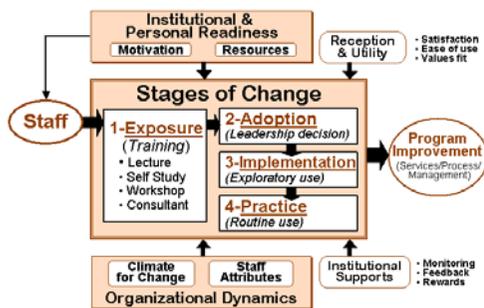
Figure 1: Sequential influences of organizational functioning and staff attributes on stages of program change (14).

The exposure of one health concept could be done by socialization, training, lecturing, self studying, holding workshops, and/or by being a consultant. Beside imposing change with a top down approach, the zoo's change should be balanced with a bottom up approach by engaging people who impacted by it (11). Setting broad goals should be performed and working through the details together should be conducted. In order to perform the institutionalize change, committed people were needed because they were engaged in the process.

The adoption of one health concept should be performed by leadership decision making approach. This implementation

should be done through exploratory use and its practice should be a part of routine use activity. Why the keyword of internalization and implementation of one health concept is performing organization's culture (11). In line with performing organization's culture, the process of exposure, adoption, implementation, and practice, could be used as lesson learned for developing program improvement of one health leadership (14).

Leadership approach

The role of leaders in creating an organisation's culture could not be neglected, because they are able to destroy it as well. When contemplating about a change, the leader must think carefully about the organisation's culture and how well the proposed change fits with its norms, values and attitudes. The organizational culture was not the mission or vision statement but the underlying soul of an organization—unspoken values, rules and traditions. To create and implement sustainable change, the agent of change must focus on the organisation's culture, reward systems, leader behaviours, and organizational design. Mike and Slocum (2003) suggest that the exploration of top management answers these following questions:

1. How do people in this workplace accomplish their work?
2. Who succeeds in the organization and who doesn't?
3. How and when do people interact with one another? Who participates?
4. What kind of work styles are valued in this workplace?
5. What is expected of leaders in the workplace?
6. What aspects of performance discussed most in performance reviews?

This leadership approach was also needed to develop soft skill and its deliverable substance, especially to accelerate motivation and performing agent of change (11).

Veterinary approach

In the implementation of one health concept, multiple approaches can be used on

change management of zoo. Beside leadership approach, veterinary approach has a unique position. Veterinary approach was needed, because wild life animals have unique characters to be handled (15). For example, if they are getting sick, the clinical signs would be noted when the illness was really severe. This clinical sign of animal was nature and really different compared to domestic animals. In this concern, the implementation of one health concept as described above should be performed. Based on this result, relevant veterinary suggestion should be done for educating stakeholders, planning the program, and participating in one health leadership.

CONCLUSION

Keywords yielded from the analysis of zoo vision and mission statement could be used as a bridge to initiate zoo change management towards one health leadership. In this concern, an understanding of zoo's role as repositories of knowledge in one health issues would be used as important tools and models for the implementation of one health concept.

Multiple approaches could be used to implement one health concept. However, leadership approach and veterinary approach had unique position in initiating zoo change management. Leadership approach was needed to create organization's culture. Veterinary approach was needed to fit organization's culture with daily activities that related to the implementation of one health concept. In this concern, veterinary leadership could be developed as an effective approach to engage one health leadership.

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Antimicrobial Resistance of *Escherichia coli* Isolated from Broiler at Rajshahi Region, Bangladesh

Muha. AjijurRahman Al Azad[1,*] Ruhul Amin[2] Mst. IsmatAra begum[3] Maximilian Baumann[4] Kannika Na Lampang[5,6] Hafez Mohamed Hafez[7]

[1] *Joint Master Course in Veterinary Public Health; (MVPH) of Freie Universitaet Berlin, Germany and Chiang Mai University, Thailand*

[2] *Scientific Officer, Bangladesh Council for Scientific and Industrial Research, Rajshahi; Bangladesh*

[3] *Associate Professor. Department of Animal Husbandry and Veterinary Science; Rajshahi University, Bangladesh*

[4] *Faculty of Veterinary Medicine, Freie Universitaet Berlin, Germany*

[5] *Department of Veterinary Public Health and Bio Science, Faculty of Veterinary Medicine, Chiang Mai University, Thailand*

[6] *Veterinary Public Health Centre for Asia Pacific (VPHCAP), Faculty of Veterinary Medicine, Chiang Mai University*

[7] *Institute of Poultry Diseases, Faculty of Veterinary Medicine. Freie Universitaet; Berlin, Germany*

Corresponding author; Email: razad80@gmail.com; r_azad80@yahoo.com

ABSTRACT Antimicrobial resistance, a major, health problem for both humans and animals throughout the world, is leading to treatment failure after administering antimicrobial drugs. This study was conducted from December 2012 to May 2013 to determine the prevalence of antimicrobial resistance of *Escherichia coli* in live broilers in Rajshahi district of Bangladesh. Five Upazilla were selected randomly and 200 cloacal swab samples of live birds were collected from 50 different broiler farms (four samples from each farm). A flock was classified as resistant to antimicrobials if one of the four samples showed resistance to any of the nine antimicrobials tested for. Isolation of *E. coli* was done by conventional microbiological methods followed by biochemical identification. A total of 200 *E. coli* isolates were collected and tested for resistance to nine antimicrobial agents (ampicillin, erythromycin, tetracycline, gentamicin, ciprofloxacin, levofloxacin, trimethoprim-sulfamethoxazole, colistinsulphate, and streptomycin). Antimicrobial resistance tests were performed by following the standard disc diffusion technique mentioned by the Clinical and Laboratory Standards Institute (CLSI-2011). Results showed that all isolates are multi-drug resistant (≥5 antimicrobial agents) and all were 100% resistant to tetracycline, erythromycin, streptomycin, ampicillin, trimethoprim sulphamethoxa-zole and ciprofloxacin. The highest sensitivity pattern of 73.5% of the isolates was determined for colistinsulphate followed by gentamycin (49%), and levofloxacin (17%).Results suggest that a high resistance of *E. coli* to antimicrobials exerts a threat to the poultry industry at Rajshahi area in Bangladesh, so raising awareness about proper administration of antimicrobials in broiler farms is crucial from an animal production and veterinary public health point of view.

KEYWORDS: *E. coli*, Antimicrobials, Resistance, Broiler, Bangladesh

INTRODUCTION

Antimicrobial resistance is becoming a global threat all over the world and due to the rapid increasing nature of this problem; the World Health Organization (WHO) recommended a global surveillance system in

veterinary and human medicine (1). The theme of the World Health Day 2011 proposed by WHO was "Antibiotic resistance: No action today, no cure tomorrow" and it was selected to create mass awareness among the world population. Anti-microbial abuse is considered to be the most vital selecting force to antimicrobial resistance of bacteria (2, 3).

Escherichia coli, a common microbial inhabitant of gastrointestinal tract of poultry and human being including other animals but may become pathogenic to both (4, 5). Most of the *E. coli* strains are nonpathogenic and may serve as indicator of fecal contamination in food. About 10-15 % of intestinal coliforms are opportunistic and pathogenic in nature (6). Pathogenic *E. coli* can cause a variety of lesions in immunocompromised hosts and in poultry. Diseases caused by *E. coli* are sometimes severe. Sometimes even lethal such as meningitis, endocarditis, urinary tract infection, septicemia, epidemic diarrhea of adults and children (7) and yolk sac infection, omphalitis, cellulitis, swollen head syndrome, coligranuloma, and colibacillosis in poultry (8).

Antimicrobial treatment is considered the most important issue that promotes the emergence, selection and spread of antimicrobial resistant microorganisms in both veterinary and human medicine (9, 10). Many studies have shown the spread of antimicrobial resistance from animals to humans (11-15). Acquired multi drug resistance to antimicrobial agents may lead to illness, death, and increased healthcare costs if it occurs due to treatment of infections caused by *E. coli* (16).

Bangladesh has a large amount of poultry production. In 2011, there were over 115,000 farms producing approximately 170 million broilers and layers (17). There is no detailed information on antimicrobial resistance in animals or humans ecospheres in Rajshahi area. Hence, the present study was designed to isolate *E. coli* strains from fifty different broiler farms of Rajshahi district in Bangladesh for assessing their susceptibility and resistance patterns to some selected antimicrobials.

MATERIALS AND METHODS

Study design

The study is a cross-sectional survey conducted from December 2012 to May 2013.

Study site

Rajshahi is a divisional city as well as a big district with a population of 2.2 million. It is located in the western part of Bangladesh bordering India to the west. It plays an important role in the food industry as the number of households involved in animal food production was 388767 (18). Out of these, 660 broiler farms produced 1,980,269 broilers in 2011 (19). Products are both locally consumed and exported to nearby districts. Antimicrobial use has been reported to be huge (Survey data). Major types of antimicrobials include ciprofloxacin, enrofloxacin, colistin sulphate, doxycycline and levofloxacin.

Sampling technique

From the list of broiler farms available at the Rajshahi district Livestock Office (19), 50 farms were selected by multistage random sampling method. Selection criteria included: at least 500 broilers in the farm, safety from political unrest and accessibility by paved road. Four broilers were randomly selected from the same farm and cloacal swabs were carried out. All swabs were kept in transport media and sent to the laboratory as soon as possible on the same day.

E. coli isolation

On the day of arrival at the laboratory, the cloacal swabs from the broilers were streaked on Mac Conkey agar (Merck) and incubated aerobically at 37ᵒC for 24 hours. Lactose fermenting colonies were then streaked on Eosin Methylene Blue (Merck) agar and incubated for 24 hours at 37ᵒC. Colonies that produce greenish metallic sheen were isolated on Nutrient Agar (Merck) (20, 21). From the Nutrient agar *E. coli* isolates were inoculated in half-strength Nutrient Agar (Merck) (incubated at 37ᵒ C for 24 hours) and stored at 4ᵒC for further identification.

Biochemical identification

Suspected colonies were confirmed as *E. coli* by negative gram's stained rods with positive glucose/lactose fermentation, gas

production and absence of H_2S production in Triple Sugar Iron agar (Merck), positive Methyl Red test and negative Vogues Proskauer test in Methyl Red VogesProskauer media (Merck), Indole production in Motility Indole Lysine media (Oxoid), and negative reaction in Urea Agar (Himedia) and Simmon's Citrate agar (Oxoid) (22). Besides *E. coli* confirmatory test (EC), gas production in Durham tube and Trypton Water (TW) test, red ring after adding covac's reagent were also done for further identification.

Antimicrobial resistance testing

Antimicrobial resistance tests were performed by standard disc diffusion technique (CLSI-2011). The selection criteria of antibiotics testing discs depended on the regularly use of antimicrobials in the broiler farms, potential public health importance and recommended from the guideline of antimicrobial susceptibility testing from CLSI (2011). Resistance testing discs contained ampicillin (10 µg), colistin (10 µg), gentamicin (10 µg), streptomycin (10 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), erythromycin (15 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), tetracycline (30 µg) (Oxoid). The isolates were considered resistant if the diameter of inhibition zone was less than or equal to the resistance breakpoint provided by CLSI guidelines. Quality control of diameters of inhibition zone against the level of resistance was based on *E. coli* (ATCC-25922) as a reference.

Data management and statistical analysis

Data processing has done by computer using Epidata version 1.3.2.1 (The Epidata Association, Odense, Denmark) and R software version 3.0.1 (R, Free Foundation for Statistical Computing, Boston, USA).

RESULTS

Number of E. coli isolates

Among 200 specimens collected from 50 different farms all were found *E. coli* positive (Table-1). From all positive samples we collected 200 isolates for further analysis and preserved them in half strength nutrient agar at 4°C.

Of the isolates, 100% were resistant to six antimicrobials named ampicillin, tetracycline,

Table 1: Sources and characteristics of samples.

No.	Name of upazilla	No. of farms from where samples taken	No. of samples taken	No. of samples in which E. coli were recovered	No. of E. coli isolates
1	Durgaour	09	36	36	36
2	Sadar	09	36	36	36
3	Godagari	09	36	36	36
4	Mohanpur	15	60	60	60
5	Tonore	08	32	32	32
	Total	50	200	200	200

Table 2: The sensitivity, intermediate resistance and resistance of *E. coli* isolates against the tested antimicrobials.

Name of Agents	Susceptible		Intermediate		Resistance	
	n	%	n	%	n	%
Colistin	147	73.5	0	0	53	26.5
Gentamycin	98	49	30	15	72	36
Levofloxacin	34	17	36	18	130	65
Ciprofloxacin	0	0	64	32	136	68
Ampicillin	0	0	0	0	200	100
Streptomycin	0	0	0	0	200	100
Erythromycin	0	0	0	0	200	100
Trimethoprim + Sulphamethoxazole	0	0	0	0	200	100
Tetracycline	0	0	0	0	200	100

Table 3: Major resistance Patterns of *E. coli* isolates (N=200).

MDR Patterns	(n)	%
CT + CIP	(30)	15
CT + CN	(22)	11
CT + CIP + LEV	(20)	10
CT + CN + LEV	(13)	6.5
CT + CIP + CN	(12)	6
CT + CIP + CN + LEV	(7)	3.5
CT + CIP + CN + LEV + S	(7)	3.5
CT + CIP + CN + LEV + S + SXT	(7)	3.5
CT + CIP + CN + LEV + S + SXT + E	(7)	3.5
CT + CIP + CN + LEV + S + SXT + E + AMP	(7)	3.5
CT + CIP + CN + LEV + S + SXT + E + AMP + TE	(7)	3.5

streptomycin, ciprofloxacin, erythromycin and trimethoprim - sulphamethoxazole. The highest sensitivity showed by colistin sulphate (73.5%), followed by gentamycin (49%) and levofloxacin (17%) respectively (Table- 2).

There were several resistant patterns and the major resistant patterns are showed in the table 3.

DISCUSSION

The prevalence of *E. coli* in 100 % of cloacal swab samples in the present study was higher than the previous records of Akond et al., (23) in Bangladesh, which were 66% only. Our study clearly indicates a higher resistance rate than previous study by others.

Many studies on antimicrobial resistance were performed in Bangladesh at different times in different region but none in Rajshahi area. M. Hossain et al., (24) from Bangladesh reported 91.42% isolates were resistant to erythromycin and 62.85% to ampicillin but in our study all isolates are resistant to both of these drugs. In contrast to our study, Nazir et al., (25) reported higher sensitivity to ciprofloxacin. In another study conducted in Bangladesh by Hashem et al., (26) claimed 100% sensitivity to colistin sulphate and here it is only 73.5% indicating an abuse of colistin. Our results support the study by Al-Ghamdi et al., (27) who showed (99.1%) resistance to tetracycline in Saudi Arabia. All isolates of this present study exhibited multiple resistances to more than six antibiotics. Similar findings on multiple drug resistance of *E. coli* strains have been reported from Bangladesh and other parts of the world (11, 27, 28).

The higher resistant rate may be due to an indiscriminate use of Antimicrobials. 80% (40/50) farmers used antimicrobials as preventive treatment. Due to the direct influence of dealer (Chick, feed and drug seller) farmers are practicing indiscriminate administration of antimicrobials. ciprofloxacin, enrofloxacin, colistinsulphate, doxycycline, trimethoprim+sulphamethoxazole, levo-floxacin are the major antimicrobials used by the farmer (Based on survey).

Antimicrobials are available and can be purchased without prescription from a veterinarian.Dueto indiscriminate exploitation of antimicrobial agents, high incidence of multi drug resistance may ultimately replace drug sensitive microorganisms from antimicrobial saturated environments (29).

CONCLUSION

The study suggests that the prevalence of resistant *E. coli* from live broiler is quite significant in respect of indiscriminate use of antimicrobial drugs. A comprehensive antimicrobial drug administration monitoring system should be urgently devised and implemented.

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First Reported Prevalence and Antimicrobial Resistance of *Campylobacter* spp. in Fresh Chicken Meat in Nueva Ecija, Philippines

Fredelon Bunnao Sison[1,2,*] Warangkhana Chaisowwong[3,4] Suruda Tiwananthagor[3,4] Duangporn Pichpol[3,4] Kannika Na Lampang[3,4] Greta Gölz[5] Thomas Alter[5]

[1] *Joint Master Course in Veterinary Public Health (MVPHCAP) of Freie Universitaet Berlin and Chiang Mai University, Thailand*

[2] *College of Veterinary Science and Medicine, Central Luzon State University, Nueva Ecija, Philippines*

[3] *Department of Veterinary Biosciences and Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand*

[4] *Veterinary Public Health Centre for Asia Pacific (VPHCAP), Faculty of Veterinary Medicine, Chiang Mai University*

[5] *Institute of Food Hygiene, Freie Universitaet, Berlin, 14163 Berlin, Germany*

*Corresponding author; Email: fredbsison@yahoo.com

ABSTRACT This study was conducted to determine the prevalence and to semi-quantify *Campylobacter* spp. on chicken breast skin samples at four selected local wet markets and to determine antimicrobial resistance patterns of the *Campylobacter* isolates. Fifty seven out of the 120 samples tested were confirmed to be positive for *Campylobacter* spp. The estimated prevalence was 47.5 (95 % CI: 38.66-56.72). Out of the 57 samples tested, 54.39% (n=31) were identified to be *C. coli* and 45.61% (n=26) *C.jejun*i. Among the four local wet markets, highest prevalence was determined in Cabanatuan with 35.09 %. Almost 53% of the samples tested positive for *Campylobacter* spp. had a contamination of MPN = ∞ (LCL 580/g) (1). With regards to degree of resistance to five antibiotics, out of the 44 isolates tested, 77.27% were resistant to ampicillin which is being the highest, followed by ciprofloxacin 70.45%, tetracycline 54.55%, erythromycin 20.25% and gentamicin 11.36%, respectively. Moreover, 81.82% (n=36) of the isolates were resistant to at least one antimicrobial agent. For two antimicrobial drugs, there were 13.64% (n=6), 38.64% (n=17) for three drugs, 13.64% for four drugs and 6.89% (n=3) were resistant to all five antimicrobial drugs. A similar trend of increasing pattern of multi-resistance was observed in the country and other countries where use of the antimicrobial drugs was moderately unrestricted in both humans and animals. Such high prevalence of *Campylobacter* spp. contamination in chicken meat at retail suggests the need of sanitary handling of poultry meat. Based on these data, we strongly suggest good and efficient intervention measures at slaughterhouses to minimize fecal contamination of broiler skin and decrease cross-contamination.

KEYWORDS: *Campylobacter spp., prevalence, antimicrobial resistance*

INTRODUCTION

Poultry meat is often contaminated with *Campylobacter*. Contaminated poultry and poultry meat is thought to be the major source of human campylobacteriosis (2, 3). *Campylobacter* organisms can be transferred from the intestines of an infected bird to its carcass during slaughter. Most cases of campylobacteriosis are linked with eating raw or undercooked poultry meat or from cross-

contamination of other foods. Most people who become sick with campylobacteriosis get diarrhea, cramping, abdominal pain, and fever within two to five days after exposure to the organism(4). Despite the zoonotic importance of this pathogen, few studies on *Campylobacter* were yet conducted in the Philippines (5, 6).

As of January, 2012, the country's broiler population was estimated at 57.28 million birds. Around 26.66% of the total inventory was in Region III (Central Luzon) which is the top producing region of chicken meat in the country (7). Yet, in this region no studies were conducted on *Campylobacter* prevalence in broiler flocks or broiler meat so far. In contrast, *Campylobacter* spp. contamination was observed in neighboring countries such as, Japan, Thailand and Vietnam where they reported prevalences of 60%, 52% and 31%, respectively, in poultry meat at retail (8-10).

Campylobacter with resistance to antimicrobial agents have been observed in both developed and developing countries (11, 12). However, in developing countries, where the use of antimicrobial drugs in humans and animals is moderately unrestricted, higher rates of enteric infections with antimicrobial-resistant bacteria could be detected (13).

The aims of this study were:(i) to determine the prevalence and to identify *Campylobacter* spp. by standard microbiological culture method, biochemical test, and multiplex PCR, (ii) to determine the semi-quantitative load of *Campylobacter* spp., and (iii) to determine antimicrobial resistance patterns of the *Campylobacter* spp. isolates from chicken meat at four local retail wet markets in Nueva Ecija, Philippines.

MATERIALS AND METHODS

From January to April 2013, 120 samples of fresh breast meat of chicken with skin were purchased from four wet markets representing four districts of the province of Nueva Ecija, namely: Cabanatuan City, San Jose City, Gapan City and Guimba. Fifteen samples were collected twice from the same chicken meat retail stalls from each wet market using random sampling. For enrichment, by performing aseptic technique, 15g of skin from each sample were removed and blended in a sterile stomacher bag with one hundred twenty milliliter of prepared Bolton broth (Oxoid, UK) with supplement (Oxoid, UK) and 5% sterile lysed horse blood, for two minutes to homogenize. Ninety milliliter from the initial suspension was transferred to sterile plastic bag. This corresponded to 10g of the test portion which also corresponded to 10^1. Ten milliliter of the initial suspension was transferred to a culture tube. This corresponded to 10^0. A 10-fold dilution series up to 10^{-4} from 10^0 dilutions were prepared by transferring 1 ml to tubes containing 9ml Bolton broth. A quantity of 1 ml from the highest dilution was discarded. The dilutions corresponded to $10^{-1}, 10^{-2}, 10^{-3}$ and 10^{-4}. Then, all Bolton broths were incubated for 48 hours at 42°C in microaerobic conditions (CampyGen, Oxoid, UK). For each enriched Bolton broth, one loop (approximately 10 μl) was streaked on modified charcoal-cefoperazonedeoxycholate -agar (mCCD agar; Oxoid, UK) and the plates were then incubated at 42 °C for 44 to 48 hours in microaerobic conditions. *Campylobacter* enumeration was done in all samples. The plates were examined for typical or suspect colonies of *Campylobacter* spp. For confirmation, at least one colony considered to be typical or suspected as being *Campylobacter* spp. was taken, streaked on Columbia blood agar (Columbia agar; Oxoid, UK) plates and incubated at 42 °C for 44 to 48 hours in microaerobic conditions. Pure cultures were examined on a glass slide with cover slip using a microscope for morphology and motility. For the detection of oxidase, a portion of a well-isolated colony was taken from each individual Columbia agar plate and streaked on filter paper moistened with the oxidase reagent. The appearance of a mauve, violet or deep blue color within 10 seconds indicated a positive reaction. Semi-quantification and microbiological identification of *Campylobacter* spp. were performed according to ISO/TS 10272-3:2010 (E). Identified *Campylobacter* spp. were confirmed, verified and differentiated into *C.*

jejuni and *C. coli* by multiplex polymerase chain reaction (mPCR). The mPCR was performed according to Wang et al. (2002) using three pairs of specific primers designed to identify the genes *hipO* from *C. jejuni*; *glyA* from *C. coli*, and the internal control 23S rRNA (14). Forty four out of the 57 confirmed *Campylobacter* spp. strains were tested for antimicrobial resistance. *Campylobacter* spp. isolates grown on Columbia blood agar plates for 24-48 hours were suspended into 1 ml sterile distilled water until a 0.5 McFarland turbidity was reached. Each suspension of *Campylobacter* isolates was streaked onto a Mueller-Hinton blood agar (MHBa) plate using a sterile cotton swab (Kirby-Bauer method)(15). Five antimicrobial discs were used for sensitivity testing, namely: ampicillin (10µg), ciprofloxacin (5µg), erythromycin (15µg), gentamicin (10µg), and tetracycline (30µg) (all OXOID, UK). The five discs where aseptically placed on the agar surface with sterilized forceps. Each disc was placed with the distance of approximately 12-15 mm from each other. Plates were incubated at 42 ˚C for 24 h under microaerobic condition. After incubation each discs was examined for the absence or presence of a growth inhibition zone which was attained by measuring the diameter. The isolates were characterized as susceptible, intermediate or resistant according to breakpoints of disk diffusion method for *Campylobacter* spp. provided by Clinical and Laboratory Standards Institute (CLSI) (16). All laboratory works were conducted at the Department of Animal Health Laboratory, Philippine Carabao Center (Gene Pool) and Molecular Biology Laboratory, College of Veterinary Science and Medicine, Central Luzon State University, Science city of Munoz, Nueva Ecija, Philippines.

Descriptive statistics were done for the estimation of prevalence, 95% confidence interval and antimicrobial resistance pattern. Semi-quantification result was interpreted based on the table provided by ISO(1).

RESULTS

Using both standard culture method (17) and multiplex-polymerase chain reaction, 57 out of the 120 samples tested were confirmed to be positive for *Campylobacter* spp. The estimated prevalence was 47.5 (95 % CI: 38.66-56.72). Out of the 57 positive samples, 54.39% (n=31) were identified to be *C. coli* and 45.61% (n=26) *C. jejuni* (Table 1).

Table 1: Prevalenceof *Campylobacter* spp. in chicken breast skin collected at four wet markets in Nueva Ecija.

No. of positive samples	No. of C. jejuni isolates (%)	No. of C. coli isolates (%)
57	26 (45.61%)	31 (54.39%)

Among the four local wet markets, the highest prevalence was recorded in Cabanatuan with 35.09 %, followed by Gapan 26.32%, San Jose 24.56% and Guimba 14.04%, respectively (Table 2).

Table 2: Distribution of *Campylobacter* spp. in positive chicken breast skin at retail.

Location	N	Positive	Prev %	95% CI	
Cabanatuan	30	20	66.7	47.18	82.71
Gapan	30	15	50.0	31.29	68.70
San Jose	30	14	46.7	28.34	65.67
Guimba	30	8	26.7	12.27	45.88
TOTAL	120	57	47.5	38.31	56.81

Almost 53% of the samples tested positive for *Campylobacter* spp. had a contamination of Most Probable Number (MPN) = ∞ (LCL 580/g) (1). Semi-quantification results on *Campylobacter* spp. on chicken meat at retail is presented in the figure below (Figure 1).
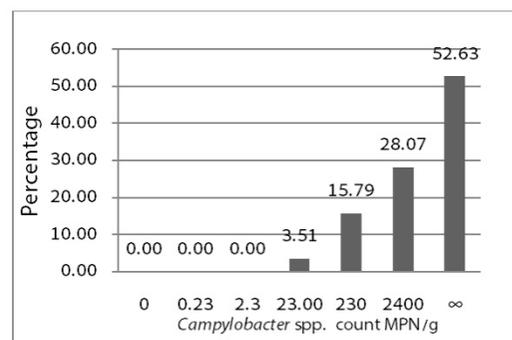
Figure 1: Percentage of *Campylobacter* spp. contamination on chicken meat at retail.

With regards to degree of resistance to five antibiotics, out of the 57 isolated *Campylobacter* spp., 44 isolates were tested. Of these, 77.27% were resistant to ampicillin, followed by ciprofloxacin70.45%, tetracycline 54.55%, erythromycin 20.25% and gentamicin 11.36%, respectively.
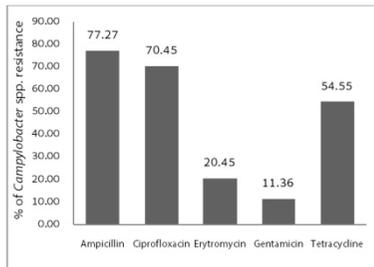
Figure 2: Percentage of *Campylobacter* spp. resistant to antimicrobial drugs.

Moreover, 81.82% (n=36) of the isolates were resistant to at least one antimicrobial agent. For two antimicrobial drugs, there were 13.64% (n=6), 38.64% (n=17) for three drugs, 13.64% for four drugs and 6.89% (n=3) were resistant to all five antimicrobial drugs. Overall, 8 different patterns were observed. Below is a table showing the pattern most frequently observed (Table 3).

Table 3: Most frequent multi-drug resistance pattern of *Campylobacter* spp. isolates.

No. of Antimicrobial drug	Most frequent pattern (n)
Two	Amp+Cip(6)
Three	Amp+Cip+Tet(15)
Four	Amp+Cip+Gen+Tet(4)
Five	All Antimicrobial drugs (3)

Note: Amp=Ampicillin; Cip=Ciprofloxacin; Tet=Tetracycline; Gen=Gentamicin.

DISCUSSION

The prevalence of *Campylobacter* spp. on chicken meat at retail in our study was 47.5 %. This was comparable to a study in Thailand conducted in poultry meat at retail that showed a *Campylobacter* prevalence of 52% (9). A study in Vietnam also reported *Campylobacter* prevalence of 31% in poultry meat at retail (10) which was also close with our result. *Campylobacter coli* was the most commonly determined *Campylobacter* species in this study which was similar with the previous study in the Philippines (5). In Thailand and South Africa, *C. coli* was also the leading *Campylobacter* species isolated from retail market (8). Nonetheless, in Hanoi, Vietnam, the most frequently isolated *Campylobacter* in chicken meat at retail was *Campylobacter jejuni* followed by *Campylobacter coli* (10). Cabanatuan, being the capital of the province of Nueva Ecija, was more likely to be contaminated with *Campylobacter* spp. probably due to various sources of chicken meat supplying it compared with the others which have less sources of suppliers.

The result of antimicrobial drug resistance were comparable to a study conducted in the Philippines (18) for all antimicrobial agent tested except for gentamicin which was 91.7%. Likewise, for the isolates tested, most common combination of multidrug resistance was to ampicillin, tetracycline, and ciprofloxacin (38.64%). This is similar to the result of a study in Thailand where they also tested the same 5 antimicrobial drugs and get the combination of multidrug resistance to ampicillin, tetracycline, and ciprofloxacin being the highest (12).

CONCLUSION

Such high prevalence of *Campylobacter* spp. contamination in chicken meat at retail suggests the need for sanitary handling of poultry meat. We strongly suggest good and efficient intervention measures at slaughterhouses to minimize fecal contamination of broiler skin and decrease cross-contamination (12). With antimicrobial resistance of isolates, a similar trend of increasing pattern of multi-resistance was observed in the country and other countries where the use of antimicrobial drugs was moderately unrestricted in both human and animals.

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Prevalence and Antimicrobial Susceptibility of *Listeria monocytogenes* in Fresh Poultry Products in Bandung, Indonesia

Yoni Darmawan Sugiri[1,*] Josef Kleer[2] Greta Gölz[2] Tongkorn Meeyam[3,4] Warangkhana Chaisowwong[3,4] Thomas Alter[2]

[1] Joint Master Course in Veterinary Public Health, Freie Universität Berlin and Chiang Mai University, Thailand, and Balai Pengujian dan Penyidikan Penyakit Hewan dan Kesmavet (BP3HK), Dinas Peternakan Provinsi Jawa Barat, West Java, Indonesia
[2] Institute of Food Hygiene, Department of Veterinary Medicine, Freie Universität Berlin, Germany
[3] Department of Veterinary Biosciences and Veterinary Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Thailand
[4] Veterinary Public Health Centre for Asia Pacific (VPHCAP), Faculty of Veterinary Medicine, Chiang Mai University, Thailand
*Corresponding author; Emails: yoni_dvm@yahoo.com, yonidvm@gmail.com

ABSTRACT The purpose of this study was to determine the prevalence and the number of *Listeria monocytogenes* in 184 samples of fresh poultry products sold in traditional markets and supermarkets in Bandung, West Java, Indonesia, and also to determine the antimicrobial resistance pattern and molecular characterization of the isolated *L. monocytogenes* strains. The samples were analyzed following ISO 11290-1: 1996 and ISO 11290-2: 1998. Disc diffusion method was applied for antimicrobial susceptibility test and multiplex polymerase chain reaction was applied for molecular serotyping of the isolated *L. monocytogenes*. Overall *L. monocytogenes* was detected in 15.8 % of the fresh poultry products, with prevalence of 15.2 % and 16.3 % for traditional markets and supermarkets samples respectively. There was no significant difference between traditional markets and supermarkets regarding the prevalence and the count number of *L. monocytogenes*. The average *L. monocytogenes* count in poultry products sold in traditional markets was 1.08 log cfu/g, and 1.03 log cfu/g for supermarkets. The contamination level of *L. monocytogenes* was < 10 cfu/g in 87.5 %, between 10 and 100 cfu/g in 9.8 %, between 100 and 1000 cfu/g in 2.2 %, and > 1000 cfu/g in 0.5 % of the samples. All 29 isolates in this study belong to the molecular serogroup IIb comprising the serovars 1/2b, 3b and 7. 27.6 % (8/29) isolates were resistant to at least one of ten antimicrobials tested, and 72.4 % (21/29) of the isolates were susceptible to all antimicrobials tested. Among 29 isolates, 17.2 %, 6.9 %, 6.9 % and 3.4 % were resistant to penicillin, ampicillin, erythromycin, and a combination of ampicillin and penicillin respectively. The results of this study reveal that there is wide spread cross contamination and emerging antibiotics resistant of *L. monocytogenes* isolated from fresh poultry products sold in study area. There are strong needs to improve this condition by improving good hygienic practices and prudent use of antimicrobial drugs at all level of poultry production chain.

KEYWORDS: *Listeria monocytogenes, Poultry products, Antimicrobial susceptibility, Serotyping, Multiplex PCR*

INTRODUCTION

Listeria monocytogenes has been largely studied in the last decades due to its importance as food borne human pathogen with high mortality rate on susceptible hosts (1). *L. monocytogenes*is a psychrotrophic pathogen and can grow at refrigerator temperatures. Minimum pH for growth on foods is 4.39 and it can grow under aerobic, microaerobic, anaerobic as well as under vacuum condition (2). *L. monocytogenes* can be found on soil, water, fresh vegetables, and intestinal contents of birds, fish, insect and other animals (3). *L. monocytogenes* can also be found in the processing environments because of poor hygiene practices in the premises (4). Listeriosis is a severe foodborne disease caused by *L. monocytogenes.* This disease has been studied and documented in the developed or industrialized countries such as in Europe and North America, and sometimes sporadic cases and irregular outbreaks of human listeriosis have been detected in developing countries (5). More than 80 % of human listeriosis cases are caused by serovars 4b, 1/2a, 1/2b and 1/2c (6). The main risk groups for listeriosis are pregnant women and their fetuses, new born children, immune-compromised, and old people (7). There are no data or information concerning the prevalence, the quantitative load, antimicrobial resistance pattern and serotypes of *L. monocytogenes* isolated from fresh poultry products in Indonesia, and only limited information regarding this disease in humans neither in animals. Therefore this study was conducted to determine the prevalence, the quantitative load, the antimicrobial resistance pattern and to characterize the *L. monocytogenes*, and also to compare the prevalence and quantitative load between traditional markets and supermarkets.

MATERIALS AND METHODS

Sample collection

A total of 184 fresh poultry products (chicken) composed of 92 samples from 12 traditional markets and 92 samples from 12 supermarkets were collected in Bandung, Indonesia between November 2012 and February 2013. All samples were taken by convenient random sampling, and transported to the laboratory aseptically in cool boxes. The samples were analyzed immediately, If the samples were not analyzed on the day of arrival at the lab, they were kept in a refrigerator (2-6°C) for not more than 24 hours before analysis.

Identification of L. monocytogenes

The isolation and identification of *L. monocytogenes* was done according to ISO 11290-1: 1996. Ten gram of each sample (chicken skin from neck and breast) were enriched in 90 ml half Fraser broth (Merck, Germany), and incubated aerobically at 30°C for 24 h. 100 µl of enriched sample were transferred to 10 ml Fraser broth, and incubated aerobically at 37°C for 48 h. The enriched samples in Fraser broth then were streaked onto Chromocult® Listeria selective agar (Merck, Germany) and PALCAM agar (Merck, Germany), and incubated aerobically at 37°C for 24-48 h. Suspected *L. monocytogenes* colonies were sub-cultured on Tryptone Soya Yeast Extract agar plates (Merck, Germany) for confirmation by biochemical and CAMP tests. All *L. monocytogenes* strains isolated in this study were stored in brain heart infusion broth with 20 % glycerol and kept at -70°C for further studies.

Enumeration of L. monocytogenes

The enumeration of *L. monocytogenes* was done according to ISO 11290-2: 1998. Ten gram of each sample (chicken skin from neck and breast) were homogenized with half-Fraser broth without the addition of selective agents, in order to revitalize stressed listeriae, the homogenate was allowed to rest for 1 hour at room temperature before plating on the agar. 1 ml of homogenate was spread onto Chromocult® Listeria selective agar and PALCAM agar, and incubated aerobically at 37°C for 24-48 h. Suspected *L. monocytogenes* colonies were counted and 3-5 colonies per plate were sub cultured on Tryptone Soya Yeast Extract agar plates for confirmation by biochemical and CAMP tests.

Antimicrobial Susceptibility test

All isolated *L. monocytogenes* strains were tested by the standard disc diffusion method described by the Clinical and Laboratory Standards Institute 2012 (8) on Mueller Hinton agar (Merck, Germany) incubated at 37°C for 24 h. The antibiotic discs that were used in this study were: ampicillin [10 µg], neomycin [10 µg], erythromycin [15 µg], penicillin [10 µg], ciprofloxacin [10 µg], streptomycin [10 µg], sulphametoxazole-trimetophrim [23.75-1.25 µg], kanamycin [30 µg] tetracycline [30 µg] and gentamycin [10 µg] (OXOID®, UK). *Staphylococcus aureus* ATCC 25923 was used as control strain.

Molecular serotyping of L. monocytogenes isolates

Strains were recovered by streaking on 5 % sheep blood agar and Trypticase Soy Yeast Extract agar and were grown overnight at 37°C. The colonies were scraped and suspended in 500 µl sterile 1x Tris-EDTA mixed and boiled for 10 minutes to extract the DNA (9). Then the samples were centrifuged and the supernatant was divided into aliquots and stored at -20°C until used for PCR. The serotyping by molecular serogroup was carried out using two multiplex PCRs according to Doumith *et al.*, 2004 (10) and Kerouanton *et al.*, 2010 (11) with some modification.

For the multiplex PCR a total of 25 µl reaction mixture were composed of 2.5 µl 10×buffer, 0.5 µl MgCl$_2$ [25mM], 0.5 µl dNTPs [10mM each], 0.1µl Taq-DNA Polymerase (Faststart, Roche, Mannheim, Germany) [5U/µl], template DNA 0.5 µl, 2.4 µl of following primer pairs (Table 1): 0.4 µl *LMO0737* [50 pmol/µl], 0.8 µl*LMO1118* [50 pmol/µl], 0.4 µl*ORF2819* [50 pmol/µl],0.4 µl *ORF21110* [50 pmol/µl], 0.2 µl *PRS* [50 pmol/µl] and 0.2 µl *LIP* [50 pmol/µl], and 18.5 µl sterile distilled water with the amplification conditions: initial denaturation at 95°C for 4 min. followed by 35 cycles with 94°C for 30 s, annealing at 58°C for 50 s, extension at 72°C for 1 min, and a final extension at 72°C for 10 min.

For the *fla*A PCR, a total of 25 µl reaction mixture were composed of 2.5 µl 10×buffer,

2.5 µl MgCl$_2$ [25mM], 0.5 µl dNTPs [10mM each], 0.1µl Taq DNA Polymerase (Faststart) [5U/µl], template DNA 0.8 µl, 0.82 µl of following primers pairs (Table 1): 0.8 µl *fla*A[50pmol/µl] and 0.02 µl *PRS* [50pmol/µl], and 17.78 µl sterile distilled water with the amplification conditions: initial denaturation at 95°C for 3 min followed by 40 cycles with 94°C for 30 s, annealing at 61°C for 40 s, extension at 72°C for 1 min, and a final extension at 72°C for 7 min.

Table 1: Primer pairs used in this study.

Gene target	Primer sequence (5'-3')	Product size
LIP/ *prf* A	Forward: GATACAGAAACATCGGTTGC Reverse: GTGTAATCTTGATGCCATCAGG	274 bp
Lmo0737	Forward: AGGGCTTCAAGGACTTACCC Reverse: ACGATTTCTGCTTGCCATTC	691 bp
Lmo1118	Forward: AGGGGTCTTAAATCCTGGAA Reverse: CGGCTTGTTCGGCATACTTA	906 bp
ORF2819	Forward: AGCAAAATGCCAAAACTCGT Reverse: CATCACTAAAGCCTCCCATTG	471 bp
ORF2110	Forward: AGTCGACAATTGATTGGTGAA Reverse: CATCCATCCCTTACTTTGGAA	597 bp
Prs	Forward: GCTGAAGAGATTGCGAAAGAAG Reverse: CAAAGAAACCTTGGATTTGCGG	370 bp
fla A	Forward: TTACTAGATCAAACTGCTCC Reverse: AAGAAAAGCCCCTCGTCC	538 bp

Data analyses

Data analysis was done using "R" statistical and data analysis free software. Descriptive statistics were used to describe the result of prevalence and enumeration analysis. The prevalence of *L. monocytogenes* between traditional markets and supermarkets were compared using chi square statistical test, and for *L. monocytogenes* enumeration data were compared using two samples independent t-test. For the antimicrobial susceptibility test, it was counted how many isolates are resistant against the antibiotics or antimicrobials used in the study. All isolated *L. Monocytogenes* strains were serotyped and serogrouped based on the results of mPCR.

RESULTS

Identification of L. monocytogenes

There was no statistically significant difference (p > 0.05) between traditional markets and supermarkets regarding the prevalence of *L. monocytogenes* on the fresh poultry products. *L. monocytogenes* was detected and isolated from 14 out of 92 samples from traditional markets (15.2 %; 95 % Confidence Interval (CI): 7.88 – 22.6), while

15 out of 92 samples from supermarkets were positive for *L. monocytogenes* (16.3 %; 95 % CI: 8.76 – 23.85). The prevalence for all the samples was 15.8 % (29/184) at 95 % CI (10.49 – 21.02) as shown in Table 2.

Table 2: Prevalence of *L. monocytogenes* in traditional and supermarkets in Bandung, Indonesia.

Type of Market	No. of Positive Samples	No. of total samples	Prevalence (%)	95% CI
Traditional Markets	14	92	15.22	7.88 – 22.56
Supermarkets	15	92	16.30	8.76 – 23.85
Total	29	184	15.76	10.49 – 21.02

Enumeration of L. monocytogenes

The average *L. monocytogenes* count of traditional markets was 1.08 log cfu/g, and the average *L. monocytogenes* count of supermarkets was 1.03 log cfu/g (Table 3). There was no statistically significant difference (p > 0.05) between the *L. monocytogenes* count of traditional markets and supermarkets.

Table 3: Descriptive statistic of *L. monocytogenes* counts in Bandung, Indonesia.

Market Type	No. of sample	Mean	Median	SD[a]	Min	Max	Rang
Traditional markets	92	1.08	0.95	0.39	0.95	3.10	2.1!
Supermarkets	92	1.03	0.95	0.23	0.95	1.98	1.0?
Total	184	1.06	0.95	0.32	0.95	3.10	2.1!

[a]SD= Standard Deviation

There were 87.5 % (161/184) of the samples had *L. monocytogenes* counts less than 10 cfu/g, 9.8 % (18/184) had *L. monocytogenes* counts between 10 and 100 cfu/g, 2.2 % (4/184) had *L. monocytogenes* counts between 100 and 1,000 cfu/g and 0.5 % (1/184) had *L. monocytogenes* counts higher than 1,000 cfu/g. The distribution of *L. monocytogenes* enumeration data from all samples (184 samples) are presented in Table 4.

Table 4: Enumeration data of *L. monocytogenes,* in Bandung, Indonesia.

L. monocytogenes Counts Range (cfu/g)	Traditional Markets (n=92)	Supermarkets (n=92)	Total (n=184)
< 10	80 (87 %)	81 (88 %)	161 (87.5 %)
10-100	7 (7.6 %)	11 (12 %)	18 (9.8 %)
100-1000	4 (4.3 %)	0 (0 %)	4 (2.2 %)
1000-10000	1 (1.1 %)	0 (0 %)	1 (0.5 %)

Antimicrobial susceptibility test

From a total of 29 isolates of *L. monocytogenes* analyzed, eight (27.6 %, 95 % CI: 11.3 – 43.9) were resistant to at least one of ten antimicrobials tested, and 21 (72.4 %, 95 % CI: 56.1 – 88.7) were susceptible to all antimicrobials tested. Five out of 29 isolates (17.2 %, 95 % CI: 3.5 – 31.0) were resistant to penicillin, 2 (6.9 %, 95 % CI: 1.2 – 20.2) were resistant to ampicillin, and 2 (6.9 %, 95 % CI: 1.2 – 20.2) were resistant to erythromycin, while one out of 29 isolates (3.4 %, 95 % CI: 0.2 – 15.3) was resistant to penicillin and ampicillin (Table 5).

Table 5: Result of antimicrobials susceptibility test of *L. monocytogenes*.

Antimicrobials agent (µg)	Resistant		Intermediate		Susceptible	
	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
AMP (10 µg)	2	6.9 (1.2 – 20.2)	0	0 (0 – 9.8)	27	93.1 (79.8 – 98.9)
N (10 µg)	0	0 (0 – 9.8)	0	0 (0 – 9.8)	29	100 (90.2 - 100)
E (15 µg)	2	6.9 (1.2 – 20.2)	0	0 (0 – 9.8)	27	93.1 (79.8 – 98.9)
P (10 µg)	5	17.2 (3.5-31.0)	0	0 (0 – 9.8)	24	75.9 (60.3-91.4)
CIP (10 µg)	0	0 (0 – 9.8)	0	0 (0 – 9.8)	29	100 (90.2 - 100)
S (10 µg)	0	0 (0 – 9.8)	0	0 (0 – 9.8)	29	100 (90.2 - 100)
SXT (25 µg)	0	0 (0 – 9.8)	0	0 (0 – 9.8)	29	100 (90.2 - 100)
K (30 µg)	0	0 (0 – 9.8)	0	0 (0 – 9.8)	29	100 (90.2 - 100)
TE (30 µg)	0	0 (0 – 9.8)	0	0 (0 – 9.8)	29	100 (90.2 - 100)
CN (10 µg)	0	0 (0 – 9.8)	0	0 (0 – 9.8)	29	100 (90.2 - 100)
AMP + P	1	3.4 (0.2 – 15.3)	0	0 (0 – 9.8)	28	96.6 (84.7 – 99.8)

n: number of isolate, AMP: ampicillin, N: neomycin, E: erythromycin, P: penicillin, CIP: ciprofloxacin, S: streptomycin, SXT: sulphamethoxazole/ trimethoprim, K: kanamycin, TE: tetracycline, CN: gentamycin.

Molecular serotyping of L. monocytogenes isolates

In all 29 isolated *L. monocytogenes* strains the *prs, prf*A and *orf2819* gene was detected by first PCR (Figure. 1). In none of all 29

isolates the *fla*A gene was detected by the second PCR (Figure. 2). From the results of the first and second mPCR assay, all *L. monocytogenes* isolates from this study belong to the molecular serogroup IIb, comprising the serotypes 1/2b- 3b- and 7.
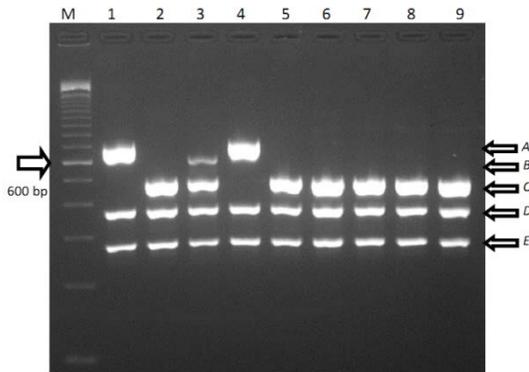
Figure 1: First multiplex PCR results. M: DNA marker 100 bp, Lane 1-4: positive control (serotype 3a, 1/2b, 4b, 1/2a), lane 5-9: *L. monocytogenes* isolates from the study, A: *lmo0737* (691 bp), B: *orf2110* (597 bp), C: *orf2819* (471 bp), D: *prs*(370 bp), E: *lip* (274 bp).
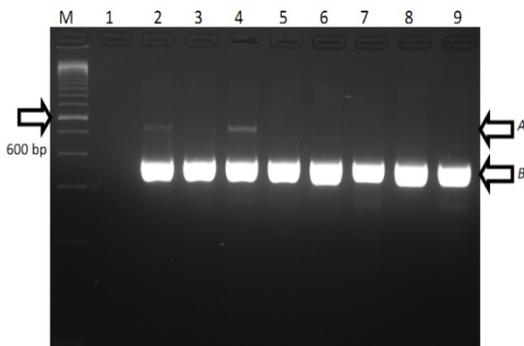
Figure 2: Second multiplex PCR (*fla*A PCR) results. M: DNA marker 100 bp, lane 1: negative control, lane 2-4: positive control (serotype 3a, 1/2b, 1/2a), lane 5-9: *L. monocytogenes* isolates from the study, A: *flaA* (538 bp), B: *prs*(370 bp).

DISCUSSION

The prevalence of *L. monocytogenes* from fresh poultry products in this study was relatively similar to the prevalences described in Thailand (13.9 %), Pakistan (17.5 %), and France (15.5 %) (12, 13, 14). There are many factors which can contribute to the contamination of fresh poultry products. *L. monocytogenes* can be found easily in the environment. It is resistant to various environmental conditions which allow longer survival under adverse conditions than most other non-spore forming food borne bacteria (15). Several studies show that *L. monocytogenes* can be found easily in poultry slaughterhouse or processing plants (16). Even though the prevalence and enumeration results of *L. monocytogenes* between these two types of markets showed no statistically significant difference, the contamination level of *L. monocytogenes* in traditional markets is slightly higher than in supermarkets. There were five samples from traditional markets with level of contamination higher than 100 cfu/g, whereas the samples from supermarkets showed level of contamination lower than 100 cfu/g. In susceptible peoples, the infective dose for *L. monocytogenes* is maybe less than 1,000 cells (15). The results of this study indicate that the fresh poultry products which were contaminated by *L. monocytogenes* higher than 100 cfu/g which is derived from the traditional markets have the potential to infect humans, especially those in high risk groups such as young children, the elderly, pregnant women, and those with immune problems (YOPI). These results show that the hygienic practices are one of many factors which can contribute to the level of *L. monocytogenes* contamination in the fresh poultry products at the selling point (16). Therefore need to increase public awareness to always cook all fresh poultry products properly before consumption, to decrease the chance of getting infected by food borne pathogen such as *L. monocytogenes.*

Although the incidence of antibiotic resistance of *L. monocytogenes* isolates in this study is still relatively low, it presents an evidence of the emergence of multi-resistant *L. monocytogenes* strains which can be a threat to public and animal health. We found that among 29 isolates, 17.2 %, 6.9 %, 6.9 % and 3.4 % were resistant to penicillin, ampicillin, erythromycin, and a combination of ampicillin and penicillin respectively. The resistance of *L. monocytogenes* strains against penicillin, ampicillin and erythromycin was also reported in the others studies, these studies showed that the resistance rate were 2.3 %, 9.2 % and

1.9 % (17), 64.3 %, 32.1 %, 57.1 % (18), 22.5 %, 23.7 %, 12.1 % (19) and 3.73 %, 1.98 %, 0.4 % (20) against penicillin, ampicillin, and erythromycin respectively. The antimicrobial resistance of *L. monocytogenes* strains isolated from fresh poultry products in this study may caused by uncontrolled and imprudent use of antimicrobial agents by farmers and their excessive use in poultry farms to control diseases in Indonesia, especially in West Java (21). Resistance of *L. monocytogenes* against antibiotics is influenced by many factors. Several studies described that conjugation of enterococcal and streptococcal plasmids and transposons which are carrying antibiotic resistance genes can be transferred to *Listeria* sp. and between species of *Listeria,* it is known that *L. monocytogenes* is frequently found in the digestive tract of humans and animals where many species of enterococci and streptococci harboring conjugative plasmids and transposons are found in very high numbers (22).

The most common serotype isolated from foods and food processing environments is 1/2a followed by 4b, 1/2b, 4c and 1/2c (23). All strains isolated in this study belong to serogroup IIb comprising serotype 1/2b-3b-7. By means of genetic ribotyping classification methods, *L. monocytogenes* was divided into three lineages: lineage I consists of serotypes 1/2b, 3b, 4b, 4d, and 4e, lineage II is composed of serotypes 1/2a, 1/2c, 3a, and 3c, and lineage III of serotypes 4a and 4c. Lineage I has the highest pathogenic potential and is mainly involved in epidemic outbreaks. Lineage II has intermediate pathogenic potential and is possibly involved in sporadic outbreaks, while Lineage III has low pathogenic risk and rarely causes human infection (7).

CONCLUSION

The results of this study reveal that there is wide spread cross contamination of *L. monocytogenes* in fresh poultry products sold in Bandung, Indonesia. Although the contamination level of *L. monocytogenes* on fresh poultry products was relatively low, the existence of this human pathogen must be considered as having considerable public health impact especially for those people who belong to the high risk group for listerial infection such as young, old, pregnant and immuno-compromised people (YOPI). This study also found that all isolates belong to the molecular serogroup IIb comprising serotype 1/2b-3b-7 which are categorized as highly pathogenic and involved in most of epidemic outbreaks. In addition to the public health impact, some of those isolates show antimicrobial resistance against antimicrobial drugs commonly used in human and veterinary medicine. There are strong needs to improve this condition by improving good manufacturing practices, good hygienic practices, good retailing practices and prudent use of antimicrobial drugs at all level of poultry production chain. These problems also deserve more attention from all stakeholders including the government as regulator.

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First Results from a Microbiological Assessment of Commercial Poultry Feeds Distributed in Nepal

Anand Kumar Singh[1,*] Mukul Upadhaya[2] Kannika Na Lampang[3] Warangkhana Chaisowwong[3,4] Hafez Mohamed Hafez[5]

[1] *Joint Master Course in Veterinary Public Health of Freie Universitoet, Berlin and Chiang Mai University, Thailand*

[2] *Department of Veterinary Public Health, Ministry of Agriculture, Nepal*

[3] *Departments of Veterinary Biosciences, Faculty of Veterinary Medicine, Chiang Mai University, Thailand*

[4] *Veterinary Public Health Centre for Asia Pacific (VPHCAP), Faculty of Veterinary Medicine, Chiang Mai University*

[5] *Institute of Poultry Diseases, Department of Veterinary Medicine, Freie Universität Berlin, Germany*

*Corresponding author; Email: anandsingh85@daad-alumni.de,anandsingh85@yahoo.com

ABSTRACT Feed production is a major component of the poultry industry because almost 80% of total cost of production goes to feed but less attention has been paid to the role of bacterial contamination of feed with regards to human food borne illness. This study aimed to identify the level of microbiological and fungal contaminations of commercial poultry feeds distributed in Nepal. The study included all registered pellet feed mills located throughout the country. Overall 130 pellet feed and 50 mash feed samples were collected and analyzed. The laboratory methods for microbiological and fungal analyses were performed on the basis of ISO Standards 4833:2003 Aerobic plate count (APC), ISO 21528-2:2004 (*Enterobacteriaceae*), ISO7954:1987 (yeast and moulds) and ISO 6579:2002 (*Salmonella*). The feed samples were examined for total aerobic plate count, total *Enterobacteriaceae* count, yeast and mould counts and detection of *Salmonella* spp. The microbiological analysis showed an average total aerobic plate count of 5.19 ± 0.81 log cfu/g of feed followed by total *Enterobacteriaceae* counts of 3.82 ± 0.78 log cfu/g and total yeast and mould counts of 3.89 ± 0.63 log cfu/g. In comparing the level of contamination between 13 feed companies, no statistically significant differences were found for APC. However, there were significant differences observed between feed companies with regards to *Enterobacteriaceae* counts ($p = 0.0183$) and yeast and moulds counts ($p = 1.04 \times 10^{-8}$). From this study we also ascertained that the investigation of *Salmonella* spp. in pellet feed did not obtain any positives whereas in mash feed 38% (n=50) of the samples were positive for *Salmonella*spp. In conclusion, the feed were found to be contaminated and could pose a potential health risk to poultry and humans.

KEYWORDS: *APC, Enterobacteriaceae, Yeast and Mould, Salmonella, Feed and Nepal*

INTRODUCTION

Poultry production in Nepal has been started as commercial enterprises since the last three decades; it has become one of the main industries contributing 3-4% to the gross domestic product (GDP) of the country. The broiler industry is considered an important source of animal protein in Nepal. Feed production is a major component because commercial broilers and layers are fully raised based on commercial feed. Feed must be healthy and microbiologically safe to get an

optimal return in production, however, microbiological assessment of feed in Nepal is scarcely practiced, in feed many microorganisms can be present and directly affecting poultry some being zoonotic and result in high economic losses. The efficiency of feed utilization in the birds and the development of feed industries of Nepal are dependent upon the quality of feeds which in turn, is based on the quality of raw materials (1).

The assessment of the microbiological status is an important element in a quality assurance system during animal feed production, trade and eventually feeding (2, 3). Thus, feed is an important element of the food safety chain of animal origin. The quality and safety of food and feeds, both of animal and plant origin, is of outmost importance for industries and consumers. It is well recognized that pathogens such as *Campylobacter* spp.*, Salmonella* spp.*, Escherichia coli* and *Listeria* spp. can be transmitted along the food chain and be a source of human illness (4). Many kinds of farm animal diseases like diarrhoeal disease, fowl cholera, *Salmonellosis*, staphylococcosis, colibacilosis and listeriosis have been traced to contamination of animal feeds (5).

Compounded feeds for poultry are perishable if not properly processed, stored, transported and used. Microbial contamination is one of the major causes among many others to affect the quality of compounded feed. Although there are no obligatory microbiological criteria; some zoonotic agents in animal feeds have to be under control i.e. *Salmonella* or other agents (6). In taking into account animal health and quality of raw materials of animal origin as well as of finished feed, there is a need to establish recommended parameters for other microorganisms; this applies especially to the total count of aerobic bacteria, total count of yeast and moulds and *Enterobacteriaceae* numbers. In Nepal, there are no such data available regarding microbiological criteria of feed nor has any kind of such study been conducted.

In such situation it is necessary to have more data concerning microbiological status of feeds produced in Nepal. Taking these aspects into consideration, this study was undertaken to assess the microbiological quality of commercial poultry feed produced and distributed in Nepal.

MATERIALS AND METHODS

Sample collection

Ten samples were collected randomly during each visit to the 13 registered pellet feed mills located throughout the country resulting to a total of130 feed samples. Meanwhile, 50 feed samples also were taken as convenient sampling from 5 of the feed companies producing mash feed in Nepal.

Microbiological analysis

130 pellet feed samples were examined for the presence of *Salmonella* spp. as well as for the number of total aerobic plate count, total *Enterobacteriaceae* count and yeast and mould counts. In spite of that, 50 mash feed samples were examined but for the detection of *Salmonella* spp.

The bacteriological and mycological quality of the thirteen brands of poultry feed were assayed to establishing their autochthonous flora. The feed samples were processed on the basis of International standards ISO 6579:2002 for *Salmonella* spp., ISO 4833:2003 (APC), ISO 21528-2:2004 (*Enterobacteriaceae*) and ISO 7954:1987 (yeast and moulds). More detailed procedures for laboratory examination were as follows:

Salmonella detection: The pre-enrichment of a 25 g feed samples was transferred to 225 ml buffered peptone water (Merck, Germany) followed by homogenization. After 18 h incubation, 0.1 ml of the first enrichment was transferred to the tube containing 10 ml of RVS broth (Merck, Germany) and MKTTn (Merck, Germany) broth. Inoculated RVS broth was incubated at 41.5 ± 1℃ and MKTTn broth at 37 ± 1℃ for 24 hours. From these broths, a loop full of material was streaked onto the surface of XLD and BPLS agar (Merck, Germany). After incubation at 37 ± 1℃ for 24 hours, colonies suspected to be *Salmonella*

spp. were selected and sub-cultured for purification and for further confirmatory tests.

Enumeration of Enterobacteriaceae: For culturing, the 25 g feed samples were mixed with 225 ml of buffered peptone water. Then serial decimal 10-fold dilutions were prepared by transferring 1ml of the initial dilution into a tube containing 9 ml of maximum recovery diluents. Dilution was made up to 10^{-6}. These appropriate dilutions were cultured by spread plate technique on VRBD media (Merck, Germany). The inoculated plates were then incubated overnight at 37°C and examined for the number of colonies presumed to be *Enterobacteriaceae*. 3 to 5 presumed colonies were subcultured on nutrient agar for confirmation.

Enumeration of total plate count and yeast and moulds: 0.1 ml of the following dilutions 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} of each sample was transferred to two plates containing plate count agar (Merck, Germany)and for the number of yeast and moulds to YGC (Merck, Germany) agar. After incubation 30°C ± 1°C for 72 ±3 hours (number of total aerobic plate count) and for 5 – 7 days at 25°C ± 1°C (number of yeast and moulds), the plates were examined for the number of colonies.
Statistical analysis

All data entry, management and analysis were done in Microsoft Office Excel 2007 and descriptive statistics were used to characterize the data by using "R" software.

RESULTS

The numbers of examined samples of poultry feed and the results obtained are shown in Table 1: Out of 130 pellet feed samples examined, none of the samples yielded *Salmonella* spp. whereas out of 50 mash feed samples 19 (38%) contained *Salmonella* spp.

Table 1: Prevalence of *Salmonella* spp. in examined poultry feed samples of Nepal.

Kind of feed	Total number of samples examined	Number of positive samples (%)
Pellet feed	130	0
Mash feed	50	19 (38)

In comparing the level of contamination between 13 feed companies, no statistically significant differences were found for APC. However, there were significant differences observed between feed companies with regards to *Enterobacteriaceae* counts (p = 0.0183) and yeast and moulds counts (p = 1.04×10^{-8}).

The obtained results for quantitative methods were analyzed according to descriptive series. The data in Table 2, shows the mean with standard deviation of bacterial and fungal counts of the feed. The average of total aerobic plate count as 5.19±0.81 log cfu/g of feed followed by total *Enterobacteriaceae* counts of 3.82±0.78 log cfu/g and total yeast and mould counts of 3.89±0.63 log cfu/g were found.

Table 2: Average bacterial and fungal count of poultry feed samples of Nepal

Sample	Total Aerobic Count (log cfu/g ± SD)	Total Enterobacteriaceae count (log cfu/g ± SD)	Total Fungal Count (log cfu/g ± SD)
FM-1	4.81 ± 0.97	3.49 ± 0.89	2.94 ± 0.51
FM-2	5.19 ± 0.85	3.49 ± 0.95	3.48 ± 0.41
FM-3	5.25 ± 1.05	3.50 ± 0.64	4.19 ± 0.54
FM-4	5.34 ± 0.81	3.84 ± 0.75	3.81 ± 0.47
FM-5	5.58 ± 0.62	4.42 ± 1.04	4.02 ± 0.68
FM-6	4.99 ± 0.63	4.21 ± 1.12	3.86 ± 0.54
FM-7	4.94 ± 0.91	3.49 ± 0.60	3.63 ± 0.50
FM-8	5.42 ± 1.00	4.33 ± 0.63	3.63 ± 0.50
FM-9	4.99 ± 0.97	3.99 ± 0.54	4.38 ± 0.64
FM-10	5.11 ± 0.64	3.86 ± 0.50	4.33 ± 0.42
FM-11	5.34 ± 0.60	3.31 ± 0.32	4.03 ± 0.52
FM-12	5.29 ± 0.82	3.79 ± 0.59	4.41 ± 0.45
FM-13	5.26 ± 0.66	3.60 ± 0.64	3.88 ± 0.40
Total:	**5.19 ± 0.81**	**3.82 ± 0.78**	**3.89 ± 0.63**

The level of contamination of feed is shown in Table 3 by total bacterial and fungal counts. The total aerobic plate count (APC) of bacteria in the majority of the examined feed samples (90%) ranged from 3 log to 6 log cfu/g. However, a small number of highest values of APC (≥6 log cfu/g) were also found in feed. The numbers of *Enterobacteriaceae* in feed samples (93%) were mostly dwells between 3 log to 5 log cfu/g and 6.15% samples were higher than 5 log cfu/g. The most often stated levels of yeast and moulds in feed samples

were ranging between 1 log to 4 log cfu/g. In about every 3rdof the samples examined, the numbers of yeast and moulds counts were higher than 4-5 log cfu/g.

Table 3: Levels of contamination of poultry feed by total bacterial and fungal count.

Level of contamination	Number of samples at the examined level of contamination (%)		
	Total aerobic plate count (%)	Total Enterobacteriaceae count (%)	Number of Yeast and Mould count (%)
≤ 4 log cfu/g	10 (7.7)	77 (59.2)	77 (59.2)
4-5 log cfu/g	43 (33.1)	45 (34.6)	49 (38.0)
5-6 log cfu/g	64 (49.2)	7 (5.3)	4 (3.1)
≥ 6 log cfu/g	13 (10)	1 (0.8)	0
Total	**130 (100%)**	**130 (100%)**	**130 (100%)**

DISCUSSION

In the presented study, the microbiological quality of commercial poultry feed used in Nepal was assessed. The results obtained indicate that most of the feed samples had high bacterial counts. In Nepal until now no microbiological criteria for feed are established. According to EU and Thai guidelines, bacterial populations in feeds should not exceed 6 log cfu/g. Following the same guidelines, fungal level should not exceed 5 log cfu/g of feed and 3 log cfu/g for *Enterobacteriaceae* respectively. With regards to the aerobic bacterial counts in the present study 10% of the samples were higher than the standard values and for *Enterobacteriaceae* even 89.2% of the samples crossed the standard limit whereas for yeast and mould counts relatively few samples (3.9%) exceeded the standard.

Usually aerobic bacterium counts and total microorganism counts are fundamental as well as general hygienic criteria. These parameters inform about the microbiological quality of feeds used, the effectiveness of the production process, and the hygienic conditions during growing of plants, harvesting, processing, storage, and distribution (7). There is a popular opinion, which states that lower number of microorganisms decreases the probability of pathogen occurrence. Besides, proteolytic and lipolitic bacteria lead to a disintegration of proteins and lipids decreasing feed nutritive values. Plant materials (cereals, oil seeds, and derivatives) are the major sources of aerobic bacterial contamination of feeds (8).

This study showed further high counts of *Enterobacteriaceae* in feed which may suggest both poor manufacturing practice and contamination through handling of raw materials, during processing and/or packaging of the finished product (9). The presence of *Enterobacteriaceae* in poultry feeds and consequently in the birds poses great economic and public health concern in terms of high mortality rate in birds due to colibacillosis and transfer of zoonotic agents like *Salmonella, Yersinia,* and *Escherichia coli* (10).

The present study showed an average fungal count of 3.9 log cfu/g which strongly depends on weather conditions during the vegetative season (11). However, plant materials are the primary and most important source of fungi in feed (field fungi) and additional contamination takes place during storage (storage fungi).The presence of moulds in feeds creates a risk of occurrence of mycotoxins, which is really dangerous for animals and humans, taking into account their carcinogenic, teratogenic, and mutagenic properties (12,13).

On the other hand, the presence of *Salmonella* species may suggest faecal as well as environmental contamination. Some of these organisms are well known pathogens of birds. (14). The transmission of *Salmonella* spp. through the environment has been shown to be cyclic, and poultry feeds have historically been viewed as important factor for contamination in poultry flocks (15, 16). The occurrence of *Salmonella* spp. of 25% in poultry feed in Nigeria, of 48 feed samples taken from Owerri municipality (17). Likewise the *Salmonella* isolation rate in U.K ranged from 1.1% to 41.7% of the samples (18). Similarly the high prevalence *Salmonella* spp. were found as 65.8% in Pakistan (19) and it seems quite higher than in present study. Six isolates of *Salmonella* were recovered from 37 (16.2%) imported fish meals (20) and mash

feed containing fish, meat and bone meals mostly used for layer and breeders, were far more frequently contaminated with *Salmonella* spp. of 21% and 4% respectively (21). Broiler and layer feed is one of the important sources of farm contamination with *Salmonella* (22). But in pellet feed mills use heat treatment during conditioning and pelleting for poultry ration and its effective way of reduce pathogen. Many books and researcher suggested that heating at 70-80$^{\circ C}$ can kill the *Salmonella* and other pathogenic organism (23). This study expressed that the presence of *Salmonella* spp. in mash feed (38%)but none out of 130 pellet feed samples yield *Salmonella* spp.

CONCLUSION

The information from the present study will help to develop a database to provide basic information on the status of hygiene in feed mills and also insights into the feed making procedures in the country. A proper documentation about feed quality will form the basis for measures towards control and prevention of food borne disease in the wake of rising consumer safety. Furthermore, it is expected to contribute to the development of national microbiological criteria for the poultry feeds.

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Presence of Class I Integrons associated with Norfloxacin- and Ofloxacin-Resistant *Salmonella* from Slaughtered Pig in Chiang Mai and Lamphun, Thailand

Phyoe Thu Aung[1,2,*] Duangporn Pichpol[3] Nattawooti Sthitmatee[3] Tongkorn Meeyam[3]

[1] Master Student, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand

[2] Researcher, Livestock Breeding and Veterinary Department, Ministry of Livestock Breeding and Fisheries, Mandalay, Myanmar

[3] Department of Veterinary Biosciences and Veterinary Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand

*Corresponding author; E-mail: phyoehtun@gmail.com

ABSTRACT Salmonellosis is one of the common bacterial foodborne diseases and plays an important role on economic and public health worldwide. The objective of this study was to identify class 1 integrons and the association with norfloxacin- and ofloxacin resistance of *Salmonella* isolated from pig slaughter house in Chiang Mai and Lamphun, Thailand. A total of 208 *Salmonella* isolates were included in this study. Norfloxacin and ofloxacin resistance were identified using standard disk diffusion method, while class 1 integrons were detected by PCR. The results showed almost 0.40% (1/208) *Salmonella* isolates were resistant to norfloxacin, whereas 8.17% (17/208) was resistant to ofloxacin. Of these isolates, class 1 integrons was detected 37.01% (77/208). The presence of class 1 integrons is significantly not associated with the resistance of norfloxacin (p=0.5524) and significantly associated with the resistance of ofloxacin (p<0.05). This study suggest that ofloxacin-resistant *Salmonella* was more widely distributed than norfloxacin resistant *Salmonella* and ofloxacin-resistant *Salmonella* was mostly associated with class I integrons which could be contribute to antibiotic resistance among swine population in Chiang Mai and Lamphun provinces.

KEYWORDS: Antibiotic resistance, Class I integrons, PCR, Salmonella

INTRODUCTION

Salmonella is a family of *Enterobacteriaceae* and is the major significant foodborne disease for human (1). In US, there were 1,000 or more reported *Salmonella* outbreaks and 1 million people get sick from eating food contaminated with *Salmonella* that happened each year (2). The National *Salmonella* and *Shigella* Center, Thailand (NSSC) reports more than 100 *Salmonella* serovars isolated from various sources throughout the country each year (3). The disease easily spreads from animals and animal products to human. The common symptoms can range from bacterial diarrhea to septicemia. Due to intestinal carriage and intermittent shedding of small number of *Salmonella*, asymptomatic pigs play an important role because pork was recognized as a one major source of human salmonellosis (4). In Chiang Mai, the prevalence of *Salmonella* fecal samples in pig slaughter house was 58.2% (2002) (5) and 63% (2009) (6) respectively due to unhygienic slaughter practice and it was not easy to

determine the role of contaminated pork in human salmonellosis. Therefore, *Salmonellosis* in human and animal is a major public health problem worldwide.

With increasing occurrence of the *Salmonella* infection, antimicrobial resistance was plays an important role in treatment of *Salmonella* infection. Antimicrobial resistance, first observed in non-typhoid *Salmonella* in the early 1990s, has become major public health issue (7). A group of fluoroquinolones, norfloxacin and ofloxacin, were mostly used in both veterinary and human medicine due to resistant to chloramphenicol, ampicillin and co-trimoxazole during the late 1980s and early 1990. The widespread use of fluoroquinolones resulted in increased rate of *Salmonella* infection with reduced susceptibility to these drugs (8).

Class 1 integrons, frequently carried antimicrobial resistance determinants, have been found in both Gram-negative and -positive bacteria and integrons with the same composition and organization have been found in unrelated bacterial species and strains in geographically distinct areas. Class I integrons are often present in plasmids, transposons and inserting sequences. Class I integrons have been reported as the most common and widespread, especially in clinical settings and one of the main contributors to the problem of antibiotic resistance dissemination (3).

There were many data concerning about *Salmonella* prevalence but data concerning about class I integrons gene and antibiotic resistance *Salmonella* are limited. Therefore, the aims of this study were to identify class 1 integrons and the association with norfloxacin- and ofloxacin resistance of *Salmonella* isolated from pig slaughter house in Chiang Mai and Lamphun, Thailand.

MATERIALS AND METHODS

Salmonella isolates:

Salmonella isolates were supported by research project of Veterinary Public Health Center for Asia Pacific during April to December of 2013. A total of 208 *Salmonella* were isolated from caecal content of slaughtered pig in Chiang Mai and Lamphun provinces. They were stocked in half-strength nutrient agar and kept at 4°C.

Antimicrobial susceptibility:

Antimicrobial susceptibility testing was performed by the standard agar disk diffusion method (Kirby Bauer method) according to Clinical and Laboratory Standard Institute (CLSI 2012) (9). Agar diffusion assays were performed on Mueller Hinton agar (Merck®, Germany).Commercially prepared norfloxacin (NOR: 10 µg) and ofloxacin (OFL: 5 µg) antimicrobial disks (Oxoid, UK) were used for antimicrobial susceptibility testing. Results were interpreted as susceptible, intermediate or resistance according to criteria recommended by the CLSI.

DNA extraction:

DNA was extracted from bacterial suspension in Tryptic Soy Broth (Merck®, US) using a standard reference protocols according to Miniprep of bacterial genomic DNA from tissue phenol-chloroform method (10).

Polymerase chain reaction (PCR):

PCR was performed according (11). Integrons were detected by PCR amplification of the class 1 integrase-specific *Int1* gene (GenBank accession no. M73819). The primers were *Int1*-F (5'-TCTCGGGTAACATCAAGG-3') and *Int1*-R (5'-AGGAGATCCGAAGACCTC-3') with 592*bp*. Each 25 µl amplification reaction mixture comprised – 2.5 µl of *Salmonella* chromosomal DNA, 0.125 µM of each primer I1F and I1R, 2.5 µl of 10 x PCR buffer,2 µl of 25 mMMgCl$_2$, 0.5 µl of 10 mMdeoxynucleoside triphosphates (dNTPs), 0.2 µl of 1U of Amplitaq polymerase and 17.05 µl of sterile distilled water. The reaction condition were carried out in a Thermo cycler using the following cycling program, initial denaturation at 94°C for 3 min, 35 cycles consists of denaturation at 94°C for 45 sec, primer annealing at 62°C for 45 sec, and extension at 72°C for 1 min, with a final extension at 72°C for 10 min and hold at 4°C.

Agarose gel electrophoresis:

The PCR products were loaded in 1% w/v agarose gel for 40 min, 2.0 Amp and 80 V, and stained with ethidium bromide.

Table 1: Distribution of antibiotic resistance pattern and association with class I integrons.

Antimicrobial agents	Result of Antimicrobial Susceptibility test			Presence of class I integrons			p value
	Resistant	Intermediate	Susceptible	Resistant	Intermediate	Susceptible	
Norfloxacin	1 (0.48)	1 (0.48)	206 (99.04)	0	0	77	0.552
Ofloxacin	17 (8.17)	4 (1.92)	187 (89.90)	14	3	60	0.00006

Data Analysis:

Statistical analysis was performed by applying Microsoft Excel 2010 and subsequently analyzed using R-commander software version 3.0. Statistical significance of percentage difference between class I integrons and each antibiotic susceptibility test were evaluated by Pearson's chi-square test or Fisher's exact test whenever appropriate. For all analyses, $p \leq 0.005$ was considered to be statistically significant.

RESULTS

Antimicrobial resistance:

Table 1 describes that there was only one isolate (0.48%) among 208 isolates were resistant to norfloxacin and 99% (206/208) was susceptible to norfloxacin among a total of *Salmonella* isolates. For ofloxacin antimicrobial resistance testing, 8.17% (17/208) was resistant and 89.90% (187/208) was susceptible in all isolates. The difference between norfloxacin and ofloxacin resistant among *Salmonella* isolates was not statistically significant.
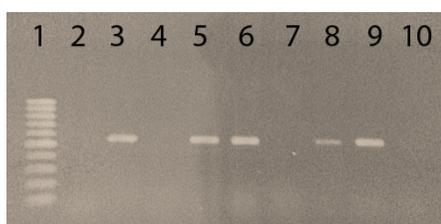
Figure 1: *PCR* amplification of class I integron s*intI* gene in *Salmonella* isolates. Lane 1: DNA ladder marker (Fermentas®, Germany) 100 *bp*, Lane 2,4,7: negative isolates, Lane 3,5,6,8,9 positive isolates with class I integrons 592 *bp*, Lane 10: negative control.

Detection of class I integrons:

This study revealed that, 77 *Salmonella* isolates (37.02%) carried class I integrase gene and possessed class I integrons (Figure 1). The presence of class I integrons was not associated with norfloxacin-resistant *Salmonella* isolates (*p*=0.55) but highly associated with ofloxacin-resistance *Salmonella* isolates (*p*<0.05) (Table 1). When compared of these two antimicrobial agents, ofloxacin was 17 % more resistance than norfloxacin.

DISCUSSION

In this study revealed that, class I integrons was found in about 37% in all isolates. This indicated that all isolates carry class I integrons was higher prevalence than the previous studies in different countries i.e., the Vietnamese, English and Dutch non-typhoid *Salmonella* isolates (12). The origin of class I integrons was not clear, but it is assumed that they were present in bacteria before "antibiotic era" (13). However, it might be the prevalence of integrons found in *Salmonella* isolates varies from country to country and depend on the origin of the isolates. There was a strong relationship between the presence of integrons and multidrug resistant *Salmonella* strains have been proven (12).

The important findings of this study was norfloxacin-resistant *Salmonella* isolates was not significantly associated with class I integrons but ofloxacin-resistant *Salmonella* was associated with the presence of class I integrons. When compared with the previous study in Lamphun province (14), resistance of norfloxacin-resistant *Salmonella* isolates was not different. But the resistance of ofloxacin-resistant *Salmonella* was higher than the previous study in Nepal (8). These findings might be due to inadequate use of ofloxacin antibiotic in pig farms and ofloxacin-resistant *Salmonella* was associated with class I integrons which could be contribute to antibiotic resistance among swine population in Chiang Mai and Lamphun provinces.

CONCLUSION

In conclusion, this study demonstrated that norfloxacin were susceptible to *Salmonella* isolates and which has been drug of choice compared with ofloxacin for salmonellosis in pig. The prevalence of class I integrons was significantly high and widespread presence in the swine population. This suggests that their acquisition may occur via the food chain and may become public health issue. Antimicrobial susceptibility testing should be performed routinely in pig slaughter house to reduce the emergence of antibiotic-resistant bacteria. The surveillance of antibiotic resistance gene combined with analysis of multilocus sequence typing (MLST) method and serotyping could be helpful for further characterization of obtained *Salmonella* isolates.

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Class 1 Integrons Presenting in Colistin-resistant *Escherichia coli* Isolated from Swine in Northern Thailand

Thanya Varinrak[1,*] Nattawooti Sthitmatee[1] Anucha Sirimalaisuwan[1] Kannika Na Lampang[1]

[1] *Faculty of Veterinary Medicine, Chiang Mai University*
Corresponding author; Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand, 50100.
Tel.: 0896368448. E-mail: thanya_var@hotmail.com

ABSTRACT Colistin-resistant *E. coli* (CREC) has been increasingly reported recently worldwide. The aims of this study were (a) to detect class 1 integrons in *E. coli* isolated from clinical swine samples in northern Thailand using polymerase chain reaction (b) to determine the trend of CREC from all *E. coli* strains and (c) to analyze the putative relationship between class 1 integrons and colistin resistance. A total of 58 *E. coli* isolated from swine were included in this study. The 58 *E. coli* strains derived from the Veterinary Diagnostic Center, Animal Health Service Center, Faculty of Veterinary Medicine, Chiang Mai University during 2010 to 2011 were subjected to determine the minimum inhibitory concentrations (MICs). The results indicated that 31/58 isolates (53.45%) carried out the class 1 integrons. In addition, totally 22/58 isolates (37.93%) were resistant to colistin. Moreover, 10 CREC strains (45.45%) carried out the class 1 integrons. The data showed the relationship between the presence of class 1 integrons and the resistance to colistin but it was not statistically significant. However, the presence of colistin-resistant *E. coli* is related to many factors. Thus, further studies are needed to provide more information about gene cassettes of class 1 integrons.

KEYWORDS: Colistin, Escherichia coli, Integrons, Swine

INTRODUCTION

Antimicrobial drug resistance has become one of the most worrisome public health problems worldwide. It is encoded by linked resistance a gene occurs on integrons, which are potentially mobile genetic elements, considered to be involved in the antimicrobial drug resistance. The exposure to antimicrobial drug resistance bacteria via the food chain is also considered a potential risk to human health (1). Given that antimicrobials are used to treat both human and animal bacterial disease, and the molecules are essential the same, it is clear that the use of antimicrobials on animals is part of this complex scenario (2).

Colistin is an antimicrobial drug belonged to the polymyxins group. The drug was discovered in 1949 and synthesized by *Bacillus polymyxa*. Nowadays, it is mainly used in human as a bactericidal agent in the digestive tract (3). Besides, it is also used to treat various disease and to increase productivity as feed additives by many swine farmers. When colistin is being used for a long time, bacteria can accumulate resistant genes on mobile genetic elements with emphasis on integrons.

Escherichia coli is a gram negative *Enterobacteriaceae* bacteria commonly found in human and animal intestinal tracts and, as result of fecal contamination or contamination during food animal slaughter, it is often found

Table 1: PCR primer sequences

Primers	Sequence (5' to 3')	Location	Annealing temperature (°c)	Cycle numbers	Product size (bp)
16S8F	AGA GTT TGA TCC TGG CTC AG	5'-CS	55	35	802
16S806R	GGA CTA CCA GGG TAT CTA ATC C	3'-CS			
intI1L	ACA TGT GAT GGC GAC GCA CGA	*intl1*	62	35	569
intI1R	ATT TCT GTC CTG GCT GGC GA	*intl1*			

in soil, water and food (4).

Integrons are genetic platforms involved in the spread of different previously captured gene cassettes that encode determinant of antimicrobial-resistance. Five classes of integrons have been defined, based on the sequence of the encoded integrases. Although class 1 and 2 are the most frequently detected in many bacterial species (2). The structure of integrons has been previously described (5, 6).

There are an increasing number of publications describing the occurrence of integrons in both human and animal bacteria, particularly in *E. coli*. However, class 1 integrons of CREC in northern Thailand has not been reported. Thus, the aims of this study were (a)to detect class 1 integrons in *E. coli* isolated from clinical swine samples in northern Thailand using polymerase chain reaction (b) to determine the trend of CREC from all *E. coli* strains and (c) to analyze the putative relationship between class 1 integrons and colistin resistance.

MATERIALS AND METHODS

Bacterial isolates

A total of 58 *E. coli* strains were collected from clinical swine samples submitted to Veterinary Diagnostic Center, Animal Health Service Center, Faculty of Veterinary Medicine, Chiang Mai University during 2010 to 2011. Samples were cultures on MacConkey agar (Merck, Germany) and lactose-positive colonies were presumptively as *E. coli*.

Colistin susceptibility testing

The minimum inhibitory concentrations (MICs) of colistin were determined by broth microdilution method according to the guidelines of the CLSI standards (7). Briefly, the direct colony suspension method was used to obtain a 0.5 McFarland adjusted inoculums, which was diluted 1: 100 to attain

the final concentration purposed by CLSI. Mueller-Hinton broth (Difco, USA) was incubated at 37°c for 20 h for further analysis of the results. *E. coli* ATCC 25922 was used as a quality control strain for broth microdilution method. A resistance cut-off value (MIC > 2μg/mL) for colistin was recommended by EUCAST(8).

DNA extraction and detection of class 1 integrons

Genomic DNA was prepared by CTAB precipitation method as described previously (9).PCR was used to detect 16s gene using the primers for 16s conserved segment positive control, 16S8F/16S806R (10) and for *int1*, IntI1L/IntI1R (11), respectively. Two microliters of the DNA was used as template DNA. The 25 μL of PCR reaction contained 1× *Taq* polymerase buffer, 1.5 mM MgCl$_2$ for 16S8F/16S806R or 2.0 mM MgCl$_2$ for IntI1L/IntI1R, 250 μM of each deoxynucleoside triphosphates (dNTPs), 1.0 μM of each primer for 16S8F/16S806R or 50 pmol of each primer IntI1L/IntI1R and 1U of *Taq* polymerase (Invitrogen, USA). PCR products were visualized on a 1% agarose gel stained with novel juice (GeneDireX, USA).

Analysis of putative relationship between class 1 integrons and colistin resistance

The data set was analysed using R software version 2.15.1 (R development Core Team, R Foundation for Statistical Computing, Vienna, Austria).Tables were created to display the frequency of CREC and integron. The association among CREC and class 1 integrons was tested by Chi-square.

RESULTS

Colistin susceptibility testing

The MIC values of all 58 *E. coli* strains were shown in Table 2. The results indicated that 22/58 strains (37.93%) were considered resistant to colistin. The MIC values of 19/22

Table 2: Distribution of MIC values of swine *E. coli* strains against colistin.

Broth microdilution	Number of strains with colistin MIC values (µg/mL)						
	0.25	0.5	1	2	4	8	16
E. coli	0	0	3	34	[19]	[3]	[0]

The values inside [ ] represent resistant strains.

Table 3: Frequency of colistin-resistance and class 1 integrons in swine *E. coli* strains.

Collection (no. of strains)	No. of colistin-resistance strains (%)	No. of strains with class 1 integrons (%)	No. of colistin-resistance strains with class 1 integrons (%)
2010 (28)	7 (25%)	11 (39.28%)	1 (3.57%)
2011(30)	15 (50%)	20 (66.66%)	9 (30%)
Total (58)	22 (37.93%)	31 (53.45%)	10 (17.24%)

strains (32.76%) were resistant to colistin at 4 µg/ml and 3/22 strains (5.17%) were resistant at 8 µg/ml. On the other hands, 37/58 strains (63.79%) were susceptible to colistin. The most frequent of MIC value is 2 µg/ml (34/37 strains; 58.62%). In 2010, 7/28 strains (25%) were considered resistant to colistin (Table 3). In 2011, 15/30 strains (50%) were considered resistant to colistin. The result also demonstrated that number of CREC was empirically increasing (Table 3).

Class 1 integrons

All 58 swine *E. coli* strains were positive for marker 16s conserved segment. Among the 58 swine *E. coli* strains, 31 strains (53.45%) were positive for class 1 integrons. In 2010, 11/28 strains (39.28%) carried out the class 1 integrons. In 2011, 20/30 strains (66.66%) carried out class 1 integrons. The result showed there was more occurrence of class 1 integrons among swine *E. coli*. Ten of 22 CREC strains (45.45%) carried out the class 1 integrons.
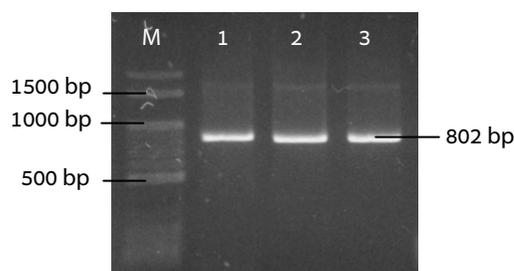
Figure 1: The 1% agarose gel electrophoresis of PCR product. The expected PCR product of 16s conserved segment was 802 bp.M : DNA Ladder. 1, 2, 3: Swine *E. coli* strains with 16s conserved segment.
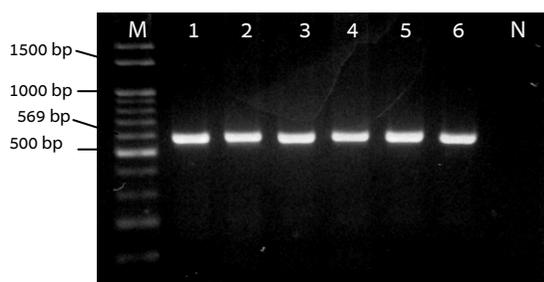
Figure 2: The 1% agarose gel electrophoresis of PCR product. The expected PCR product of *intI1* gene was 569 bp.M : DNA Ladder. 1, 2, 3, 4, 5, 6 : Swine *E. coli* strains with *intI1* gene. N : negative control.

The p-value of testing the association among class 1 integrons and CREC was greater than 0.05 (p-value=0.34). The p-value showed that the association among the presence of class 1 integrons and resistant to colistin but not statistically significant.

DISCUSSION

Colistin is an antibiotics drug that used in premedication as feed additives or therapeutics in swine farm. In this study, twenty-two strains (37.93%) were considered resistant to colistin. Percentages of colistin-resistant strains in 2011 were increasing 25% from 2010.

Morales (12) reported 8/126 strains (6.3%) of *E. coli* isolated from swine in Brazil were resistant to colistin and Boyen (13) reported 15/157 strains (9.6%) of swine *E. coli* isolated in Belgium were resistant to colistin. Moreover, our results showed higher

frequency of CREC strains from swine than the previous studies.

Integrons are mobile genetic elements thought to play an important role in the dissemination and accumulation of resistance genes in bacteria. Integrons carry one or more genes in the form of tandem gene cassettes. Transcription is initiated by a promoter sequence upstream of the gene cassettes. Each cassette is flanked by conserved sequence, which is recognized by a specialized site-specific recombination enzyme called integrase (10). The presence of integrons is strongly associated with antimicrobial resistance (14, 15). The integrons system has the ability to create novel combinations of resistance genes. Furthermore, the entire integrons element is often contained within another mobile genetic element such as plasmids and transposons, which suggested that the entire integrons elements can spread horizontally through bacterial populations.

Class 1 integrons are widely distributed in *E. coli* from human and animals (10, 14, 15, 16, 17). The previously investigations have been described the association between class1 integrons and multidrug resistance strains (6, 17). In 2011, there was more frequent of strains with class 1 integrons than 2010 (27.38%). In this study, there were 45.45% of CREC strains that carried out class 1 integrons. Percentage of colistin-resistance strains with class 1 integrons were increasing 26.43% during 2010 to 2011. The result also showed that colistin-resistance strains were increasing, indicated that using colistin as a feed additives might induce *E. coli* accumulate resistant genes and show resistance phenotypes.

CONCLUSION

The present studies showed that there were 31/58 strains (53.45%) that carried out class 1 integrons and 10/58 strains were resistant to colistin and carried out class 1 integrons. The data showed relationship between the presence of class 1 integrons and resistant to colistin but not statistically significant. However, the presence of colistin-resistant *E. coli* is related to many factors.

Thus, further studies are needed to provide more information about gene cassettes of class 1 integrons.

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Interrelationship of Livestock Associated-Methicillin-Resistant *Staphylococcus aureus* (LA-MRSA) among Pigs, Workers and the Farm Environment in Northern Thailand

Orapun Arjkumpa[1,2,3] David Love[4] Soawapak Hinjoy[5] Karoon Chanachai[6] Thomas Alter[7]
Khwanchai Kreausukon[3] Suvichai Rojanasthien[3] Prapas Patchanee[3,*]

[1] *Field Epidemiology Training Program (FETP), Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Thailand*
[2] *Veterinary Research and Development Center (Southern Region), Department of Livestock Development, Ministry of Agriculture and Cooperation, Thailand*
[3] *Department of Food Animal Clinic, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand*
[4] *Johns Hopkins Center for a Livable Future, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA Department of Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA*
[5] *Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Nonthaburi, Thailand*
[6] *Bureau of Disease Control and Veterinary Services, Department of Livestock Development, Ministry of agriculture and cooperation, Bangkok, Thailand*
[7] *Department of Veterinary Medicine, Panel "Veterinary Public Health", Institute of Food Hygiene, Free University Berlin, Berlin, Germany*
Corresponding author; Email: patprapas@gmail.com, prapas.pat@cmu.ac.th

ABSTRACT   Livestock associated-methicillin-resistant *Staphylococcus aureus* (LA-MRSA) has been emerging among pigs and pig handlers worldwide. This study aimed to determine the prevalence of LA-MRSA in pigs, workers and environment in Northern Thailand and to investigate phenotypic characteristics of LA-MRSA isolates. One hundred and four pig farms were randomly selected from the total of 21,152 pig farms in Chiang Mai and Lamphun provinces in 2012. Nasal and skin swab samples were collected from five pigs and two workers in each farm. As well, five environmental samples (pig stable floor, faucet and feeder) were collected using cotton swabs. MRSA was identified and confirmed by multiplex PCR from pooled samples of pigs, pig worker and farm environment. Phenotypic characterization of MRSA isolates were performed by Kirby-Bauer disk diffusion susceptibility test. The total of 104 pig farms and 138 workers were collected. The herd prevalence of MRSA was 8.65% (9 of 104 farms). The prevalence of MRSA in pigs, workers and the farm environment was 0.96%, 4.34% and 2.88%, respectively. Thirteen MRSA isolates were identified from eight workers, four isolates from environmental samples and one isolate from pigs. Ten of thirteen MRSA isolates were tested for antimicrobial resistance; these isolates were 100% resistant to clindamycin, cefoxitin, tetracycline, penicillin and sulfa-trimethoprim and 100% of all isolates showed multidrug resistant phenotype. This survey provided the first evidence of interrelationships for LA-MRSA among pigs, workers and the farm environment in Thailand. There was a low prevalence of MRSA in pigs, workers and the environment compared to other countries. Multi-drug resistant of MRSA isolates was observed. Further monitoring studies of MRSA in pig associated environment are required to detect changes in epidemiology and to implement effective control measures.

KEYWORDS: *Interrelationship, LA-MRSA, pig, worker, environment*

INTRODUCTION

Staphylococcus aureus is an opportunistic bacterium that is considered as microflora of human and various animals (1). It frequently colonizes in the anterior nares (2), which may cause infections when the host immune system becomes compromised. This organism developed resistance to the antibiotic methicillin (i.e., methicillin resistant *Staphylococcus aureus*; MRSA) through the *mec*A gene that is part of a large mobile genetic element (1), as first reported by Robinson and Enright in 2003 (3). MRSA has become a pathogen of increasing importance in hospitals, the community and livestock operations (4). To date, livestock associated MRSA (LA-MRSA) had been distributed worldwide, particularly among people who are involved with livestock farming (5, 6, 7). These bacteria can be transmitted to humans in close contact with MRSA colonized animals (8) meanwhile livestock, especially pigs; can serve as a reservoir for LA-MRSA (9). The prevalence of LA-MRSA among pigs, workers and the environment varies by geographic areas. The majority of strains of LA-MRSA belong to the sequence type (ST) 398 in Europe and America, while ST9 is found in Asia (5, 10, 11, 12). In Thailand, MRSA has been isolated from healthy pigs (13, 14) and pork (15). However, investigation of LA-MRSA prevalence and importance in livestock, especially from pigs in Thailand is unknown. The aims of this study were to determine the prevalence of MRSA in pig farms and farm workers in Northern Thailand as well as to investigate genotypic and phenotypic characteristics of MRSA for potential relationship between humans, animal and the farm environment.

MATERIALS AND METHODS

Study design and study population:

A cross-sectional study was conducted among pigs, workers and the environment in pig farms of Chiang Mai and Lamphun provinces of Northern Thailand in 2012. Farm operations are varied from large industrial facilities to small holding settings. Target populations of pig farms located in both provinces were 21,152 farms, based on a 2012 pig farm registry list from the Department of Livestock Development, Ministry of Agriculture, Thailand (16). Sample size was calculated from pig farms with an expected prevalence of 20% (17), accepted error of 10% and a 95% confidence level using Win Episcope 2.0. One hundred and five pig farms were determined, then proportional sampling was conducted with a 7:1 ratio of Chiang Mai farm (n=18,508) to Lamphun farms (n=2,644). This resulted in 62 farms and 53 farms sampled in Chiang Mai and Lamphun provinces, respectively, for a total of 105 farms.

Sample collection:

Demographic data of farm and information on farm management including farm type, number of pigs, herd size, period of operation, antibiotics used and personnel protective equipment used in workers were collected as well as swab samples from pigs, workers and the environment;

Pig: At each farm, groups of weaning pigs, fattening pigs and sows were sampled, if presented. From each group, nasal and skin swabs were collected from 5 randomly selected pigs by a veterinarian. The nasal and skin samples were collected from both sides of external nares and auxillary regions. Samples were collected using sterile cotton swabs. Swab samples from each group of 5 pigs were pooled and stored in Stuart transport medium and kept cool in an ice box.

Worker: Farm workers were invited to participate in the study if they work on farm at least one year. A maximum of two workers were recruited each farm. All eligible participants were asked to sign a written informed consent document. Samples from both sides of participants' external nares and the axillary regions were collected using sterile cotton swabs.

Environment: Environmental samples were collected from pig stables. Five sites including stable floor, faucet and feeder were collected by using cotton swabs. Swab samples were stored separately in transport media. All swab

samples were transported to the central laboratory, Chiang Mai University, Faculty of Veterinary Medicine within 24 hours for further investigation.

MRSA isolation and identification:

All swab samples were incubated for 48 hours at 37°C in pre-enrichment media containing tryptic soy broth with 10 ml of 10% NaCl. Then, samples were inoculated onto mannitol salt agar with 6 mg/l of oxacillin and incubated at 37°C overnight. Three suspected single colonies of *S. aureus* from each sample were selected and identified by Gram's staining with gram positive cocci and biochemical test as catalase test positive. Colonies were then re-streaked on tryptic soy agar plates overnight for colonies duplication. A coagulase test was carried out and the positive samples were further screened for methicillin resistance by disc diffusion of oxacillin 1 μg. MRSA isolates were further investigated by multiplex PCR screening for detecting of the presence of mecA gene. All MRSA isolates were kept in brain-heart infusion broth with 15% glycerol and sent for molecular testing.

Antimicrobial Susceptibility Test (AST):

AST was performed using disk diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute (18). The following disks were used including amoxicillin-clavulanic acid, cefoxitin, ceftriaxone, cephazolin, chloramphenicol, clindamycin, penicillin, cloxacillin, doxycycline, gentamycin, oxytetracycline, sulfa-trimethoprim,tetracyclineand Vancomycin.

RESULTS

Prevalence of LA-MRSA:

Isolation and identification of *S. aureus*, MSSA and MRSA are shown in Table 1. The overall MRSA prevalence at all farms was 0.96% in pigs (1 of 104 farms), 4.34% in workers (6 of 138 workers) and 2.88% in the environment (3 of 104 farms). Herd prevalence of LA-MRSA was 8.65% (9 of 104 farms). LA-MRSA isolates were found in 8 workers, 4 environmental samples and one pig. There was one farm in Chiang Mai where

LA-MRSA was isolated from both a pig and the environment. MRSA was identified in 8 of 39 farms (20.5%) in Chiang Mai and one farm (1.5%) in Lamphun.

Table 1: Prevalence of *S. aureus*, MSSA and MRSA

	N (880)	% S. aureus (n)	95% CI	%MSSA (n)	95% CI	%MRSA (n)	95% CI
Nursery							
Nasal swab	24	0.0 (0)	0.0-11.7	0.0 (0)	0.0-11.7	0.0 (0)	0.0-11.7
Skin swab	23	0.0 (0)	0.0-12.2	0.0 (0)	0.0-12.2	0.0 (0)	0.0-12.2
Fattening							
Nasal swab	65	1.5 (1)	0.0-7.3	0.0 (0)	0.0-4.5	1.5 (1)	0.0-7.3
Skin swab	69	1.9 (2)	0.2-6.8	1.9 (2)	0.2-6.8	0.0 (0)	0.0-4.2
Sow							
Nasal swab	57	0.0 (0)	0.0-5.1	0.0 (0)	0.0-5.1	0.0 (0)	0.0-5.1
Skin swab	54	0.0 (0)	0.0-5.4	0.0 (0)	0.0-5.4	0.0 (0)	0.0-5.4
Sub-total	292	1.0 (3)	0.2-2.7	5.4 (2)	3.2-8.5	0.3 (1)	0.0-1.6
Environment							
Stable floor	104	0.0 (0)	0.0-2.8	0.0 (0)	0.0-2.8	0.0 (0)	0.0-2.8
Faucet	104	2.9 (3)	0.6-8.2	1.9 (2)	0.2-6.8	1.0 (1)	0.0-5.2
Feeder	104	2.8 (3)	0.2-6.8	0.0 (0)	0.0-2.8	2.8 (3)	0.6-8.2
Sub-total	312	1.9 (6)	0.7-3.9	0.6 (2)	0.1-2.1	1.2 (4)	0.4-3.0
Worker							
Nasal swab	138	8.6 (12)	4.7-14.3	5.0 (7)	2.2-9.7	3.6 (5)	1.3-7.8
Skin swab	138	3.6 (5)	1.3-7.8	0.7 (1)	0.0-3.5	2.8 (4)	0.9-6.8
Sub-total	276	6.1 (17)	3.7-9.4	2.8 (8)	1.3-5.4	3.2 (9)	1.6-5.8

Characteristics of LA-MRSA:

Ten representative MRSA isolates were further analysed by antimicrobial susceptibility test. Sources of MRSA isolates were from workers (n=7 isolates) and environment (n=3 isolates). Susceptibility
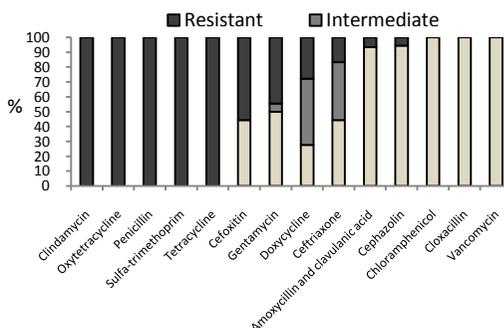
Figure 1: Antimicrobial susceptibility testing of MRSA isolates.

testing revealed 100% resistance to clindamycin, cefoxitin, tetracycline, penicillin and sulfa-trimethoprim. No resistance was observed for choramphenicol, cloxacillin and vancomycin (Figure 1). There were six different patterns of antimicrobial drug resistance in workers and environment isolates (Table 2). All isolates were resistant to at least five antimicrobials.One isolate was resistant to 11 antimicrobial drugs (worker: DA-OT-P-SXT-TE-FOX-CN-DO-CRO-AMC-KZ).

Table 2: Antibiograms of worker and the environment MRSA isolates.

Sources	Resistance profiles	#isolates (%)
Worker	DA-OT-P-SXT-TE	1 (10)
	DA-OT-P-SXT-TE-FOX	2 (20)
	DA-OT-P-SXT-TE-CN	2 (20)
	DA-OT-P-SXT-TE-FOX-CN-DO	1 (10)
	DA-OT-P-SXT-TE-FOX-CN-DO-CRO-AMC-KZ	1 (10)
Environment	DA-OT-P-SXT-TE	1 (10)
	DA-OT-P-SXT-TE-FOX	1 (10)
	DA-OT-P-SXT-TE-FOX-CRO	1 (10)

DA= clindamycin, OT= oxytetracycline, P= penicillin, SXT= sulfa-trimethoprim, TE= tetracycline, FOX= cefoxitin, CN= gentamycin, DO= doxycycline, CRO= ceftriaxone, AMC= amoxicillin-clavulanic acid, KZ= cephazolin

DISCUSSION

Findings from this study may be used as the basic information regarding the burden of MRSA associated with pig industries. The prevalence of LA-MRSA among pig farms in Northern Thailand was lower than in previous studies in other countries including 1.4% in Malaysia(12), 11.4% in China(11), 22.7% in Korea(17) 26% in Canada(19), 36% in USA(6), 39% in Netherland(5), and 49% in Germany(20) between 2008 and 2013. However, the prevalence variation is depending on many factors including geographical region, sampling methods, laboratory testing methods(21) and age of pigs tested(19). Commercial large farm size in the US and in the European countries comparing with smaller farms settings in Thailand may cause more opportunities for pathogen transfer and higher prevalence of MRSA than in this study. MRSA colonization among pigs workers in Northern Thailand was low (2.8%) comparing with other studies among pig workers in Europe (22, 23) and the USA (19). Other studies of MRSA prevalence in risk populations including slaughterhouse workers and veterinarians in Europeranged between 3% and 12.5 %(24, 25, 26). Our results revealed that pig workers in northern Thailand were at a lower risk of MRSA colonization than other countries, perhaps because of the prevalence of MRSA in pigs is lower.

MRSA was isolated from the environment in this study with the prevalence 2.88% which was lower than the results from the study in the USA (17.3%) (27).*Staphylococci* in the farming environment could serve as a source of MRSA type as it was generally easily detectable in both pig and the environmental samples (28). Our results showed that only one farm had MRSA positive samples from both pigs and the farm environment, and no farms had MRSA positive samples from both pigs and pig workers. MRSA is a human bacterial pathogen that has emerged as a major threat in both a hospital setting (as a nosocomial infection) and as a community-acquired infection for high-risk groups such as slaughterhouse workers (4). The use of antibiotics in livestock production has selected for multi-drug resistance. In this study, there were various resistance phenotype of MRSA isolates from farm workers and the environment with combined resistance to clindamycin, cefoxitin, tetracycline, penicillin and sulfa-trimethoprim, whereas other studies of MRSA-ST9 in China showed similarity of MRSA resistance patterns in workers to clindamycin, cefoxitin, tetracycline and ciprofloxacin(11). These antibiotics are commonly used in both human medicine and food animal health management. Overuse or misuse of medically important antibiotics in animals is emerging as a public health concern due to community-associated antibiotic resistant infections (29).

To our knowledge, this is the first study to demonstrate the prevalence of LA-MRSA among pigs, farm workers and the environment in Thailand. Therefore,

continuous efforts to monitor of MRSA in these populations are required for detecting changes in epidemiology and for the implementation of effective control measures in livestock and human health. Meanwhile, conducting studies in different areas in Thailand, such as the central or eastern part of the country where the highest pig population reside, should be performed. Limitation of this study were the sampling method did not use a stratified sampling technique to study a specific production system and the study was somewhat under-powered, because observed MRSA prevalence was lower than expected MRSA prevalence used in sample size calculations.

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First Findings on the Prevalence of Extended-Spectrum β-Lactamases Producing *Escherichia coli* (ESBL-producing *E. coli*) and Risk Factors in Dairy Farms in Beijing Area, China

Farong Xu[1,2,*] Uwe Rösler[3,*] Anika Friese[3] Maximilian Baumann[3] Jingyi Zhao[2] Haitao Wei[2] Xiaodong Liu[2] Khwanchai Kreausukon[4,5,*]

[1]*Joint Master Course in Veterinary Public Health (MVPH) of Freie Universitaet Berlin and Chiang Mai University, Thailand*

[2]*Beijing General Station of Animal Husbandry and Veterinary Service, Beijing, China*

[3]*Faculty of Veterinary Medicine, Freie Universitaet Berlin, Berlin, Germany*

[4]*Department of Food Animal Clinic, Faculty of Veterinary Medicine, Chiang Mai University*

[5]*Veterinary Public Health Centre for Asia Pacific (VPHCAP), Faculty of Veterinary Medicine, Chiang Mai University*

*Corresponding author; Email: xufarong@hotmail.com, uwe.roesler@fu-berlin.de, kkhwanchai@yahoo.com

ABSTRACT For the purpose of investigating the herd prevalence of ESBL-producing *E. coli* in dairy farms in Beijing area, China, a cross sectional study was conducted to detect the frequency of ESBL-producing *E. coli* in fecal samples of dairy farms Beijing area as well as to assess veterinary drug use on these farms. Fecal samples were collected and questionnaires were administered on 99 farms. Feces was diluted by Maximum Recovery Diluent (MRD) and streaked on MacConkey containing 2 mg/L cefotaxime (MAC-CTX) plates to isolate ESBL-producing *E. coli*. Isolates were confirmed by using API 20 E biochemical kits and disk diffusion tests were applied to screen and confirm for ESBLs. Data analysis was performed by using R statistic software. The results showed that in sixteen out of 99 farms (16.2%) ESBL-producing *E. coli* were found. Antimicrobial susceptibility testing was performed and among the 16 isolates, 100% were resistant to cefotaxime and ampicillin, 75% were resistant to streptomycin, 81.3% to kanamycin, 37.5% to chloramphenicol, 56.3% to gentamicin and ciprofloxacin, 50 % showed a resistance to trimethoprim, 43.8% to tetracycline, 62.5% to trimethoprim and sulfamethoxazole combination, 68.8% to sulfamethoxazole, 31.3% were resistant to nalidixic acid, and 87.5 % were multidrug resistant. The study identified that using antimicrobial contaminated milk to feed calves was associated with the detection of ESBL-producing *E. coli*.

KEYWORDS: *ESBL-producing E. coli, Prevalence, Drug resistance, Dairy farm*

INTRODUCTION

Antimicrobial resistance in bacteria has emerged as a problem in both human and veterinary medicine, and farms including dairy farms are a reservoir of ESBL-producing *E. coli*(2). The occurrence of ESBL producing *Enterobacteriaceae* in the fecal microflora of farm animals represents an obvious risk for the contamination of raw food products from animal origin(2). Therefore, ESBL-producing *E. coli* may be transmitted to human beings through raw milk or contact to the animals' direct environment such as dairy mattresses or even by the surroundings of dairy farms. Preventing, controlling and eliminating ESBL-producing *E. coli* in dairy farms is very important for the health of humans and animals, and thus, their welfare. There are no

published studies describing the herd prevalence of ESBL-producing *E. coli* and the risk factors associated in dairy farms in Beijing area. For these reasons, we carried out a study to investigate the prevalence of ESBL-producing *E. coli* in dairy farms in Beijing area, and analyzed the risk factors associated with the detection of ESBL-producing E. *coli*.

MATERIALS AND METHODS

Dairy farms, questionnaires and samples

A total of 99 dairy farms in Beijing area were selected for a questionnaire survey about farm management including the use of antimicrobials. Fecal samples were collected in recruited farms and sent within 24 h for further investigations to the lab. In each selected farm, five animals were conveniently selected and about ten grams of fresh feces were collected from each animal using sterile plastic bags. Local veterinarians administered the questionnaires when they visited the farms and collected samples in the period between November 2012 and April 2013.

Bacterial isolation and identification

ESBL-producing *E. coli* were isolated in accordance with the direct plate method described by Kreausukon(4) and simply modified by using Maximum Recovery Diluent (MRD) to dilute the fecal samples. Two g per cow (n = 5) were pooled to a sample of 10 g and homogenized in 90 mL of MRD. Aliquots of 0.1 mL were directly plated on MacConkey (MAC) agar plates and MacConkey containing 2 mg/L cefotaxime (MAC-CTX) plates. The streaked plates were aerobically incubated for 24 h at 37°C. The presumptive *E. coli* colonies were streaked on Trypticase soy agar (TSA) to get purified colonies.

One colony from each suspected sample was confirmed as *E. coli* by using API 20 E kit (Biomerieux, Craponne, France) (4). Screening and confirmatory tests for ESBLs were performed by disk diffusion tests according to the CLSI (M100-S22) recommendations.

Antimicrobial susceptibility testing

For ESBL-producing *E. coli* isolates antimicrobial susceptibility tests were performed using the agar disk diffusion method as recommended by the CLSI (1). For

each drug, it is indicated on the recording sheet whether the zone size is susceptible (S), intermediate (I), or resistant (R) based on the interpretation chart of CLSI.

Quality control and statistical analysis

E. coli ATCC 25922 was used as quality control strain for antimicrobial susceptibility tests as well as screening and confirmation tests for ESBLs. Data were processed and analyzed using Microsoft Excel and R statistic software (3). Descriptive statistics, including mean, standard deviation, median, minimum, maximum and frequency were employed as general analytic procedure to describe the overall information on the farms and antimicrobial administration status. Odds ratios and 95% confidence intervals were calculated by using epiR package.

RESULTS

Questionnaire survey

The questionnaire survey provides an overview of the herd structure as well as milk yields. The dairy farms included in the study had a median size of 287 cows, ranging from 15 to 2736 animals. The median number of calves, heifers, cows in the previous year and lactations per farm were 49, 56, 280 and 160, respectively. The median milk yield/cow and bulk milk SCC were 6100 kg and 315,000 cells/ml, respectively. The mean culling rate in the previous year was 14%, ranging from 3% to 74%.

The most common used antimicrobials on the 75 respondent farms were penicillin which was administered on 48% of the respondent farms, while cephalosporin was administered on 36.0%. The proportion of farms which had chosen Erythromycin, Gentamicin and Penicillin, Linocomycin varied from 1.3% to 4%. The indications for choosing antimicrobials included mastitis, surgery, dystocia and metritis.

Bacterial isolation and confirmation

ESBL-producing *E. coli* were isolated in 16 of 99 sampled farms by the direct plate method resulting in a herd prevalence of ESBL-producing E. *coli* of 16.2% for the investigated region. All these isolates were confirmed as *E. coli* by using API 20 E kit

(99.8%). Confirmatory tests for ESBLs were carried out by using disk diffusion method and the results show that all 16 strains demonstrated more than 5 mm increase in the zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus the zone when tested alone for cefotaxime or ceftazidime.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing results showed (Figure 1) that of the 16 isolates, 100 % were resistant to cefotaxime and ampicillin, 75% were resistant to streptomycin, 81.3% to kanamycin, 37.5 % to chloramphenicol, 56.3 % to gentamicin and ciprofloxacin, 50% showed a resistance to trimethoprim, 43.8% to tetracycline, 62.5% to trimethoprim and sulfamethoxazole combination, 68.8% to sulfamethoxazole, 31.3% were resistant to nalidixic acid, and 87.5% were multidrug resistant. None of them were resistant to amoxicillin+ Clavulanic acid.
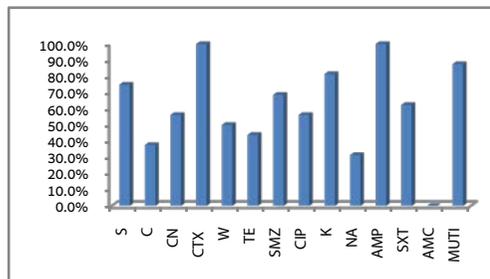
Figure 1: Proportions of susceptibility to 13 antimicrobial agents as determined by disk diffusion of 16 ESBL-producing *E. coli* strains isolated from feces obtained at sampling on a single occasion from 99 dairy farms in Beijing, China. S-Streptomycin, C-Chloramphenicol, CN-Gentamicin, CTX-Cefotaxime, W-Trimethoprim, TE-Tetracycline, SMZ-Sulfamethoxazole, CIP- Ciprofloxacin, K- Kanamycin, NA- Nalidixic acid, AMP-Ampicillin, SXT-Sulfamethoxazole + Trimethoprim, AMC- amoxicillin + Clavulanic acid.

Statistical analysis

Table 1 shows the associations of ESBL-producing *E. coli* tested positive results and risk factors including farm administration and antimicrobials using status in dairy farms. The odds ratios (OR) for different risk factors were variable. For the risk factor feeding of milk with antimicrobial residuals to calves, an odds

Table 1: Association between detection of ESBL-producing *E. coli* in fecal samples and the risk factors.

Risk factors		No. farms	Prevalence (%)	OR	95%CI of OR
Farm level	Large	28	21.4	1.65	0.44-5.75
	Small	71	14.1		
Feed Waste milk to calves	Yes	33	15.2	0.89	0.22-3.14
	No	66	16.7		
Other animals raised	Yes	25	16	0.98	0.21-3.73
	No	74	16.2		
Feed AB residual milk to calves	Yes	37	29.7	4.74	1.35-19.25
	No	62	8.1		
Rotation AB	Yes	64	12.5	0.49	0.14-1.66
	No	35	22.9		
Continue use AB until cured	Yes	90	16.7	1.59	0.19-75.64
	No	9	11.1		
Record AB usage	Yes	73	15.1	0.75	0.21-3.07
	No	26	19.2		
Using slow release AB	Yes	24	25	2.15	0.56-7.63
	No	75	13.3		
Use AB treat metritis	Yes	77	16.9	1.28	0.31-7.74
	No	22	13.6		
Use AB treat dystocia	Yes	37	24.3	2.5	0.74-8.82
	No	62	11.3		
Use AB treat cow diarrhea	Yes	53	17	1.11	0.33-3.87
	No	45	15.6		
Use AB treat surgery	Yes	44	20.5	1.68	0.5-5.88
	No	53	13.2		
Use AB treat lameness	Yes	52	17.3	1.08	0.32-3.77
	No	43	16.3		
Use AB treat calve diarrhea	Yes	68	19.1	1.96	0.48-11.65
	No	28	10.7		
Prevent disease using AB for dried cow	Yes	40	22.5	2.14	0.64-7.51
	No	59	11.9		

AB-Antimicrobials, Pos-ESBL-producing *E. coli* positive, Neg-ESBL-producing *E.coli* Negative, OR-odds ratio, CI-Confidence interval.

ratio of 4.74 (95% CI: 1.35-19.25) indicates that farms that feed antimicrobial residual milk to calves had 4.74 times the risk of detecting ESBL-producing *E. coli* compared to farms that did not feed antimicrobial residual milk to calves. For the other assumed risk factors, we did not find associations with the detection of ESBLs-producing *E. coli* in dairy farms.

DISCUSSION

Based on known published literature, this is the first study on herd prevalence of ESBL-producing *E. coli* which illustrated the risk factors associated with the detection of ESBL-producing *E. coli* in dairy farms in Beijing area, China. This study also presents an overview of the usage of antimicrobials and a description of farm management situations in this area. The study identifies risk factors for reducing the prevalence of ESBL-producing *E. coli* and the need to regulate antimicrobial drug administration in dairy farms.

There are related studies on the prevalence of ESBL-producing *E. coli* in dairy farms in European countries. The first case of ESBL-producing *E. coli* in British farm animals was found by the Veterinary Laboratories Agency

in the autumn of 2004 in Wales. Tests showed that 56% of calves were positive for these bacteria (6). Further research carried out in 2008/2009, found that 37.5% of randomly selected dairy herds in the North-West of England were found to have CTX-M ESBLs, and farms which had used modern cephalosporins in the previous year were four times more likely to be positive (5). ESBL-producing *E. coli* were isolated from bulk tank milk samples from 30% of the farms from north Germany and the detection of ESBL-producing *E. coli* was associated with the occurrence of metritis and dystocia on the farms (4).

Studies on ESBL-producing *E. coli* in animals are very limited in China. Tian*et al* reported in 2011 that the individual prevalence of ESBL-producing *E. coli* in pig farms was 9.1% (57/602)(7). Yao *et al.* reported there were big differences for drug resistance of *E. coli* between isolates from pig farms and from dairy farms. The individual prevalence of drug resistance of *E. coli* from pig farms was much higher than that from dairy farms according to a study in 2011 which is probably due to the more strict drug administration in dairy farms compared to pig farms (8).

In the present study, the herd prevalence of ESBL-producing *E. coli* from dairy farms in the region of Beijing was 16.2%. The prevalence was determined by investigation with the direct method only and with 2 mg/L CTX in MacConkey agar plates. Some existing studies utilize methods which are more sensitive such as the use of 1 mg/L CTX in MacConkey agar plates after enrichment.

The study showed that feeding of milk contaminated with antimicrobials to calves rather than discarding it represents a risk factor. This result shows that the identified risk factors related to the use of antimicrobials in farms induces the detection of ESBL-producing *E. coli*. That similar principle is reflected in previous studies on the use of modern cephalosporins(5) and for treatment of metritis and dystocia (4).

Compared to the developed countries, the use of 3rd and 4th generation cephalosporins in dairy farms in Beijing area, China started not so long ago (around 2004 and 2009, respectively). Furthermore, the dairy farms in Beijing area are very prudent in using antimicrobials to treat cow diseases due to the fact that antimicrobial residues in milk are the predominant factors for rejection when selling milk. However, the present study showed that the ESBL-producing *E. coli* are widely distributed also in the dairy farms in Beijing region.

This study aims to promote awareness among concerned stakeholders on the wide distribution of ESBL-producing *E. coli* in dairy farms in the Beijing area and to recommend a strict regulation on the use of antimicrobials especially for the 3rd and 4th generation cephalosporin in dairy farms.

CONCLUSION

The present study determines the herd prevalence of ESBL-producing *E. coli* and analyzes risk factors for inducing the occurrence of ESBSL-producing *E. coli* in dairy farms in the Beijing region. Furthermore, this study outlines first principles for preventing, controlling and eliminating ESBL-producing E. *coli* in dairy farms.

ACKNOWLEDGEMENT

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Antimicrobial Resistance Pattern of *E. coli* Isolated from Endometritis Dairy Cows

Veerasak Punyapornwithaya[1] Atcharawan Takamtha[2] Sukolrat Boonyayatra[1] Khwanchai Kreausukon[1]

[1] *Department of Food Animal Clinic, Faculty of Veterinary Medicine, Chiang Mai University*

[2] *Satellite Animal Hospital, Faculty of Veterinary Medicine, Chiang Mai University*

ABSTRACT The objective of this study was to determine the antimicrobial resistance pattern of *Escherichia coli* isolates collected from dairy cows with endometritis. Uterine discharge from 90 dairy cows, 30-90 days postpartum, with abnormal vaginal discharge was collected using transcervical guarded swab with aseptic technique. Bacteriological examination was performed under conventional aerobic conditions. Sensitivity to antimicrobial agents was tested by the disk diffusion method. Of all 120 isolates, 28 isolates were *E. coli*. Most of *E. coli* isolates were resistant to amoxycillin (32%, n=9/28) and streptomycin (28%, n=8/28). Thirteen isolates (46%) were resistant to more than one type of antimicrobial agents. Three isolates were only resistant to amoxycillin/clavulonic acid whereas 2 isolates were only resistant to streptomycin. The resistant pattern of the combination of amoxycillin/clavulonic acid, amoxycillin, ampicillin, oxytetracyclin and streptomycin were found in2 isolates. In conclusion, the study showed a moderate occurrence of multiple antimicrobial resistance of *E. coli* and some antimicrobial resistance patterns. This finding may reflect to the prudent use of antimicrobial agents in dairy farm.

KEYWORDS: *Antimicrobial resistance, E. coli, endometritis, dairy cow*

INTRODUCTION

Multiple antimicrobial resistances in bacteria populations are the world wide concern. The use of antimicrobial drugs in food animal has been shown to contribute to the increased prevalence of antimicrobial-resistance bacteria of human significance (1). Improper use of antimicrobial agents in dairy farm may associate with the increasing of multidrug-resistance bacteria. Although many studies reported the antimicrobial resistance pattern of bacteria isolated from mastitis cows, few data are available from endometritis cows. As the using of antimicrobial agents for intrauterine therapy is normally practiced for endometriris cows (2), it is important to determine the antimicrobial resistance status of bacteria in uterus. One of the most important bacteria types associated with endometritis is *E. coli*. Importantly, the microorganism is concerned for public health as *E. coli* may be transmitted to farm workers as a direct contact with the vaginal discharge, manure mixed with the discharge or other environmental sources such as cow's bedding and floor (3).

Determination of resistance pattern of bacteria is important for ongoing surveillance and for veterinarian in choosing effective antimicrobial therapy. Therefore, the aim of this study was to determine the antimicrobial resistance pattern of *E. coli* isolated from endometritis cow.

MATERIALS AND METHODS

Definition of endometritis

Cows having physical abnormal discharge at cervix and vagina were defined as endometristis cows based on the criteria described previously (4,5).

Animals

Cross bred Holstein-Frisian cows, lactation 1-6, with 30-90 days postpartum with abnormal vaginal discharge were enrolled for the study. The presence or absence of abnormal vaginal discharge was checked using a stainless steel probe with a semi-spherical rubber cup attached at one end (Metricheck™ device) by insertion of the device transvaginal and collection the mucous from vaginal tract and external os cervix. Additionally, all cows were from small holder dairy farms in Mea on district, Chiang Mai.

Collection of uterine discharge

Uterine endometrium discharged will be sampled from endometritis cows. The collection of uterine discharge was operated using technique described previously (6). Briefly, a transcervical guarded swab was used for swabbing cervical discharge. The swab comprised a 0.3 cm diameter cotton wool tip sheathed in a long sterilized stainless steel 45 cm long, 0.6 cm diameter with 1.5x0.5 cm hole at 0.5 cm from the blocked tip and wrapped with plastic breeding sheath with the hole at the same position. To prevent contamination, this guard tube was covered with sanitary sheath before inserted through the vagina. The guard tube was pushed through the sanitary sheath when it reached external os cervix. Then the guard tube was passed through the cervical canal into the lumen of the uterus guided by palpation per rectum. In the uterine lumen, the swab was extruded from the guarded tube to swab uterine discharge. Withdrew the swab into the guard tube and removed from the uterus. The swab was transferred to the transport media and cultured within 3-4 hours after collection.

Microbiological examination

Bacteriological samples were cultured for aerobic bacteria on sheep blood agar and enriched in brain heart infusion (BHI) medium for 24 h at 37°C. The broths were streaked onto sheep blood agar plates and cultured for a second time 24 h at 37°C. Bacteria were identified on the basis of the characteristics of the colony, gram stain, morphology, hemolysis, biochemical profile with triple sugar iron (TSI) agar and motility-indole-lysine (MIL) medium, and other standard tests such as citrate, catalase, urease and growth on MacConkey agar. Plates containing 1 or more colony-forming units were regarded as positive bacterial growth. Plates with more than 3 species or with mixed culture of moderate degree or higher were considered contaminated (7). The contamination plates were excluded for further test.

Antimicrobial sensitivity test

The antimicrobial sensitivity was tested by the disc diffusion method and performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines in Mueller-Hinton agar (8). The following antibacterial agents (Oxoid) were used: amoxicillin with clavulanic acid (AMC; 30 µg), amoxicillin (AML; 10 µg), ampicillin (AMP; 10 µg), cephalexin (CL; 30 µg), cefazolin (KZ; 30 µg), gentamicin (CN; 10 µg), oxytetracycline (OT; 30 µg), streptomycin (S; 10 µg) and sulfamethoxazole-trimethoprim (SXT; 25 µg). Interpretation of the test results were sensitivity (S), intermediate sensitive (I) and resistant (R) based on CLSI criteria (8).

RESULTS

Of all 120 isolates, 28 isolates were characterized as *E. coli*. Twenty out of 28 isolates (71%) were resistant to at least one type of antimicrobial agent tested. Most of *E. coli* isolates were resistant to amoxicillin and streptomycin (Figure 1). Antimicrobial resistance pattern for *E. coli* was shown in Table 1. Thirteen isolates (46%) were resistant to more than one type of antimicrobial agents. Three isolates were only resistant to amoxycillin/clavulonic acid whereas 2 isolates were only resistant to streptomycin. Interestingly, 3 isolates were resistant up to 5 antimicrobial tested.

Table 1: Antimicrobial resistant pattern of *E.coli* isolates from endometritis dairy cows.

Resistant phenotype observed	Frequency
AMC	3
CL	1
S	2
CL, KZ	1
CN, S	1
OT, S	1
AMC, AML	2
AMC, AML, AMP	1
AML, AMP, OT	1
AML, AMP,S	1
KZ, OT, SXT	1
AML, AMP, OT, S	1
AML, AMP, OT, SXT	1
AMC, AML, AMP, OT, S	2
AML, AMP, OT,S,SXT	1

AMC=amoxicillin with clavulanic acid, AML= amoxicillin, AMP= ampicillin, CL = cephalexin, CN= gentamicin, KZ =cefazolin, OT=oxytetracycline, S = streptomycin and SXT = sulfamethoxazole-trimethoprim

DISCUSSION

The main objective of this study was to determine the antimicrobial resistant pattern of *E. coli* which is considered to be an important pathogen related with public health. This microorganism may be transmitted from dairy cows to farm workers (3). In addition, the pathogen may be contaminated in raw milk and transmitted to human by raw milk consumption.

Nearly fifty percent of *E. coli* isolates were resistant to more than 1 type of antimicrobial agents suggesting that the status of antimicrobial resistances indairy production requires more attention.

Generally, *E. coli* is considered as a major pathogen causing endometritis in dairy cows(4). Previous report in Thailand indicated that most bacteria isolated from endometrium of metritis cows were most resistant to oxytetracycline (6) which is an antimicrobial agent commonly used for treating postpartum endometritis. The finding from this study was consistent with previous report as *E. coli* isolates were highly resistant to oxytetracycline (Figure 1). Furthermore, our results showed that high proportion of *E. coli* isolates (n=7; Table 1) were resistant to the combination of amoxicillin/ampicillin and other antimicrobial agents. Unlike oxyteratcycline, amoxicillin and ampicillin is not commonly used for intrauterine therapy. However, these antimicrobial agents have been commonly used in dairy farms for treatment of other diseases such as mastitis, respiratory disease and reproductive diseases. It is possible that *E. coli* isolates causing endometritis were from *E. coli* strains resistant to amoxicillin/ampicillin contaminated in environmental sources and transmitted to uterus.
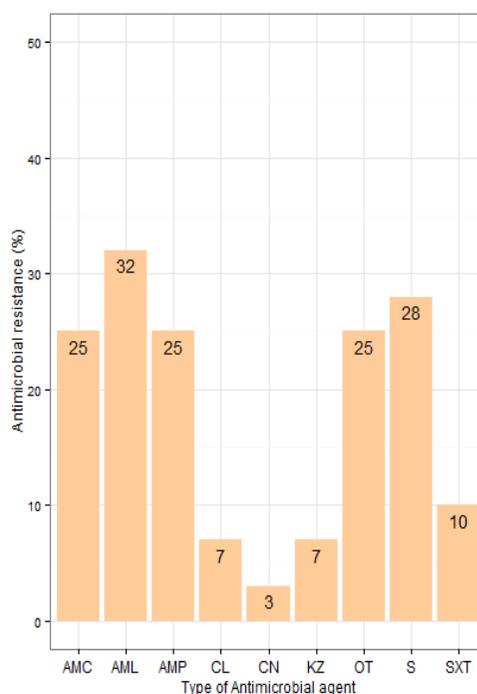
Figure 1: Antimicrobial resistance (%) of *E .coli* isolates for 9 antimicrobial agents tested. AMC=amoxicillin with clavulanic acid, AML= amoxicillin, AMP= ampicillin, CL = cephalexin, CN= gentamicin, KZ =cefazolin, OT=oxytetracycline, S = streptomycin and SXT = sulfamethoxazole-trimethoprim.

It was surprisingly that some isolates were resistant to amoxicillin with clavulanic acid whereas this antimicrobial agent is not commonly used in the study area. A further investigation should be implemented to understand more about this finding. Most of *E. coli* isolates were susceptible to gentamicin

and sulfa-trimethroprim. This finding was consistent with an investigation from our veterinarians from veterinary teaching hospital, faculty of veterinary medicine, Chiang Mai University located in the study area which indicated that these antimicrobial agents are not regularly used in the area.

A high proportion of *E. coli* isolates were resistant to more than 3 types of antimicrobial agents which may reflect to the prudent use of various types of antimicrobial agents in dairy farms. In the current study, we found 15 antimicrobial resistant patterns in *E. coli* isolates (Table 1). The most frequency pattern for multiple antimicrobial resistances was the combination of amoxicillin/clavulonic acid, amoxicillin, ampicillin, oxytetracyclin and streptomycin which was found in 2 *E.coli* isolates from dairy cows from different farm. However, the sample size was limited, thus we could not conclude that such pattern was a major pattern.

Another benefit of this study is that veterinarian has some information for choosing the effective antimicrobial agent for treatment of endometritis cows caused by *E. coli*.

Due to the difference in production system, the use of antimicrobial for each area or each country may differ. In the study area, antimicrobial agents are often used with little or no veterinary consultation. Ideally, decision making for choosing type of antimicrobials, dosage and duration of treatment should be done by veterinarians. However, dairy farmers, livestock technicians and artificial insemination persons are able to purchase antimicrobial agents by themself. Due to the limitation in education and training, the prudent use of antimicrobial agent is likely to be occurred. Because of the more antimicrobial agents used improperly, the more chances for bacteria to resist to the antimicrobial agents, strategies aimed at decreasing unintended interaction between antimicrobial agents and bacterial pathogens and treating infection with the effective antimicrobial agents should be focused. Antimicrobial uses surveillance should be done periodically to monitor the resistance rate. Education and extension supporting the optimized use of antimicrobial agents by dairy farmers is essential. Finally, the policy to control the usage of antimicrobial agents in dairy farms should be implemented.

In conclusion, this study provided updated information for antimicrobial resistance profile of *E. coli* isolated from endometritis dairy cows. The percentage of multidrug resistance bacteria was remarkable. Most of *E.coli* isolates were resistant to amoxycillin and streptomycin. The ongoing surveillance system on antimicrobial uses in dairy farms should be employed.

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Cost - Benefit Analysis of Road Safety Intervention in Banphai District KhonKaen Province

Sila Tonboot[1*] Pudtan Phanthunane[1] Jirawan Kijlertponpiroj[2] Tanapol Sonoop[3]

[1] Department of Economics, Faculty of Business ,Economics ,and Communications , Naresuan University Phitsanuloke 65000

[2] Banphai Hospital , Banphai District Khonkaen Province 40110

[3] KhonKan Highway District 3 (Banphai District) , Banphai District Khonkaen Province 40110

*Corresponding author; Email: lancelot_roundtable@hotmail.com

ABSTRACT   This study evaluated the effectiveness of street lighting installation at Banphai PTT gas station. We chose subjective method to find the fatality rate of not having light. The answer showed 8.6% of not having light the severe crash could lead to death. The cost for 2 years of the treatment program consisted of administrative cost and operational cost (meeting cost, MIS system management cost, and black spot treatment cost) and it was 158,179 Baht. This program could save 2 lives in 2 years and benefit of the program calculated by 2 lives saved multiplied by value of death from accident case(1,835,007 Baht). Then, the gross benefit multiplied by 8.6 % above. We would get net benefit and divided net benefit by cost. The result would appeared as benefit cost ratio 1.99.As a result, it was concluded that this treatment program was an effective way for the black spot solution.

KEYWORDS: *Cost Benefit Analysis, Black Spot Treatment, Banphai District*

INTRODUCTION

At the front of PPT gas station of Banphai District, there was no light in the night time and trailers parking at the pavement. These could cause many deadly accidents. From the Banphai MIS report(1)they concluded that 8 death cases happened at the area and they urgently proposed this black spot to the meeting of Banphai Security Council. The process of treatment followed and street lighting was installed after two weeks.

The purpose of this study was to analyze costs and benefits of the street lighting installation program. Instead of taking a whole portion of its benefit, we separated the causes of accidents at the black spot into fractions which we believed that those incidents were from more than a factor that caused casualties.

MATERIALS AND METHODS

Cost Estimate

The Cost Benefit Analysis method (CBA) was applied. The CBA guidelines (2, 3) recommended that administrative cost should be taken into account. After many literature reviews, cost estimating methods were different in each study. Some used the budget received to be estimated cost (4, 5) ,some used only the cost of intervention(6, 7). However there were some of studies specified their management cost and overhead cost in the cost estimation (8-10). We reviewed and defined the black spot treatment costs from its process as shown in picture below.

After the accident happened, the case would be brought to MIS meeting. They analyzed the causes of accident and proposed them to Banphai Security Council to reach the solution. If the process took a long time, the temporary solution would be proceeded. After the permanent solution Bhanphai MIS team would monitor and reported the result to the council again. Therefore, our costs of the program consisted of MIS team meeting cost ,

Banphai Security council meeting cost, MIS System management cost, temporary black spot treatment cost, and street lighting installation cost because they brought us the black spot solution. If there were no meeting of the MIS team, the process couldn't go through the treatment process.

Figure 1: Banphai MIS working process.
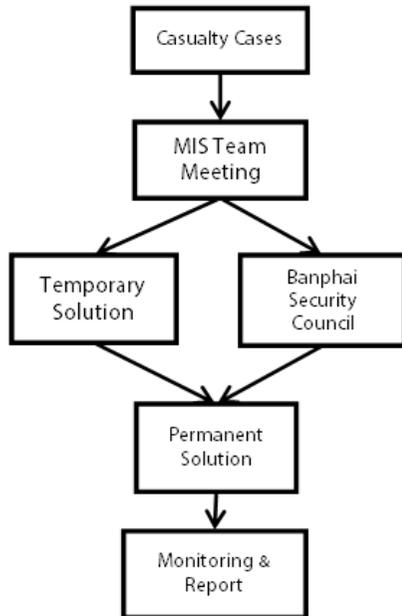
Benefit Estimate

Since, the benefit of the program was the decreased cost of death. We used the result of The Study of Traffic Accident Costs in Thailand(11),a KhonKaen province case study in 2004. The cost of death was 3,094,829 Baht a death case(11),but it included the quality of life values(11)which some guidebook recommended to be difficult things to estimate(12). The quality of life in KhonKaen province valued 1,403,581 a death case(11),so we subtracted them off from 3,094,829. Net value of cost of death from accident in KhonKaen was 1,691,248 Baht a death case. This result was adjusted to be the value in 2010 by using the Consumer Price Index(CPI) from the Bank of Thailand[1].Hence, the cost of

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[1]Consumer Price Index (2554=100), Bank of Thailand .http://www2.bot.or.th/statistics/ReportPage.aspx?reportID=409&language=th (2 May 2013)

accident values of Khonkaen province in 2010 were 1,835,007.69 Bath a death case. We used the result to multiply amount of lives saved and compared the value of death between before and after the treatment process.

To evaluate its benefit the before and after study used in the study. The data range was 2 years before and 2 years after the treatment which were October 2007 to October 2009 as before the treatment and March 2010 to March 2011 as after the treatment. The death case numbers from Banphai Police Station daily reports were 2 cases and Banphai MIS report (1) showed 8 cases before the treatment, but it indicated that they got the number from interviewing.

After the treatment there were no report of dead case from Banphai MIS team and police station. A gas station manager, a 7-11 store manager, and people who worked at the PTT gas station assured us that there was no crashing incident had ever happened again after the treatment. We could conclude that the treatment process could save at least 2 lives.

Separation of Accident Factors

While we were considering the causes of accident, we found that there were numbers of factor influenced the causes of crash. For example a drunk motorbike driver without a helmet drove fast in the PTT black spot area, then he crashed with a standing container. The driver suddenly died at the spot. This incident could not conclude that because of the only darkness brought the driver's death, but it was from alcohol, and environment also. For these reasons we developed the separation of accident causes method to filter out the only street lighting effect from three sources.

1) The Cochrane Database of Systematic Reviews provided the results of street lighting installation could decrease the fatal crashes 17 to 68 %(13),but this could not be proxy of the Banphai Area.
2) Banphai MIS report had already included the causes of accident factors from street lighting 9.3%(1),but it was the ratio of accident at the PTT gas station to all accidents in Banphai area. This numbers

did not reflect the causes of death in the black spot area.

3) Finally, we surveyed opinions of Banphai MIS team and people who used the road and lived nearby the site to be a proxy of black spot data. The 13 causes (Table1) of accident factors took from World Report on Traffic Injury Prevention (14) and the environment of the black spot analysis from Banphai MIS report(1). These 13 factors were rated in Likert scale(1-5). The main Factors were also rated by 20 members of Banphai MIS team in percentages.

Table 1: The causes of accident at the PTT gas station black spot by subjective method.

Main Factors	Average of percentage rated (1)	13 Accident Causes	Sum of Likert Scale scores (2)	Weighted accident factors (1x2=3)	Adjusted percentage (4)
Traffic Condition	15%	Traffic density	70	10.50	3.78 %
Road Condition	12%	Rough road	62	9.92	2.65 %
Road Environment	24%	Traveling in darkness	98	23.52	8.60 %
		The presence of gas station	89	21.36	7.81 %
Road users Condition	34%	inappropriate parking	104	35.36	12.63 %
		Presence of alcohol	95	32.3	11.54 %
		inappropriate speed	92	31.28	11.17 %
		Fatigue	60	20.4	7.29%
		Being a young male	73	23.82	8.87 %
		Being a vulnerable road user in residential area	57	19.38	6.92 %
		Bad driving behavior (No helmet, Using telephone)	69	23.46	8.38 %
		Poor road user eyesight	57	19.38	6.92 %
Vehicle Condition	15%	Vehicle factors	64	9.6	3.45 %
Total	100			277.91	100

The Average percentage of main factors(1)multiplied by sum of Likert scale scores(2). The results were weighted accident factors(3) and they were divided by sum of Weighted Accident factor scores(277.91). The result became adjusted percentage of accident factors(4). We used only the traveling in darkness factor to multiply the benefit. By these process we had a net benefit from lighting effect.

From three sources, each number multiplied by the benefit of street lighting installation. The results became the net benefit from the treatment program and divided by 2years total cost to get Benefit Cost Ratio (BCR).

Sensitivity Analysis

Since the lighting effect separation in the area was difficult to define. Three sources (Cochrane reviews, MIS report, Subjective method)of light effect to fatal crash were put in one way sensitivity analysis to analyze the minimum and maximum range of Benefit Cost Ratio (BCR).

RESULTS

The costs of the program per year showed in the table below.

Table 2: Cost of the treatment program.

Cost Types	Amount(Baht)
MIS team meeting cost	17,023.55
Security Council meeting cost.	8,711.78
MIS management cost	2,333.33
Temporary solution cost	12,500.00
Permanent solution cost a year	38,521.00
Total Cost	79,089.74

The treatment program took 2 weeks as it was an urgent agenda for Banphai Security Council. The meeting cost was higher than usual because the opportunity cost calculated. The temporary solution cost was land rental charged for parking area. For two year cost, total cost multiplied by 2.

After we concluded that at least 2 lives saved, this multiplied by cost of death from accident in KhonKaen(11) (1,835,007.69). We received a gross benefit of lighting installation (3,670,015 Baht). The gross benefit multiplied by 8.6 %, then the net benefit from lighting installation was 315,457 Baht. Divided net benefit by total cost we had benefit cost ratio. It was 1.99.

The lighting effect separation we chose was from subjective method. This could not reflect to the number of accident in the area. We then took one way sensitivity analysis. The number 8.6%, 9.3%(1), and 95% CI 17 to 68 pool RR 34 ,the number from Cochrane reviews(13) were used to find the minimum and maximum BCR results. The minimum BCR was 1.99 and maximum15.78.

DISCUSSION

The study aimed to evaluate effectiveness of street lighting installation. The results showed BCR 1.99 and it was an effective solution. This finding agreed with street lighting installation program in United State(15) that the cost was very low, but the different things were the nature of black spot. Most of other studies, they evaluated overviews of the state or countries(15-18), not in each spot. Moreover, they had more numerical data. This could show that the accident system monitoring of Thailand in the local area level were poor. Instead of low effectiveness of street lighting installation as our result (8.6%), the Cochrane reviews found the street lighting installation could decrease fatality rate 17-68%(13). This was not because of lacking of numerical data but quite from data management system making it difficult to use because the source of data still recorded in the book. This could caused human error in counting it. Having a good data managing system would provided accurate analysis of black spot and easy to assess the effectiveness of the treatment program.

CONCLUSION

The BCR 1.99 showed the effectiveness of street lighting installation. This could recommend that at the front of the gas station that there was no light, the lighting installation was a good accident prevention.

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Mothers' and Childs' Food Styles Comprehension in Urban and Semi Urban Area in Indonesia

Evi Martha[1] Tiara Amelia[2] Nurul Narulitasari[3]

[1] Department of Health Education and Behavior Science, Faculty of Public Health, University of Indonesia
[2] Department of Health Educationa and Behavior Science, Faculty of Public Health, University of Indonesia
[3] PDRC (Positive Deviance Resource Center), Faculty of Public Health, University of Indonesia

ABSTRACT Indonesia still has high number of undernourished children among low income communities which is mother as the one who determine food for children based on affordability, food accessibility, economic situation and social cultural. This qualitative research was conducted between November 2011 and February 2012 to explore food style among mothers and children of low income community and factors affecting it in East and West Jakarta as representative of Urban area and Bantul district as semi urban area. Data were collected through In-depth interviews with health providers (12), grandmothers (3), cadres (3), food vendors (3) and traditional birth attendant (3); and Focus Group Discussion with mothers (9 groups @ 8 mothers). Results reveal that snacking behavior has become a way of life more among mothers in Jakarta than Bantul, because of high number of food vendors, lack of kitchen ownership, cooking is less practical, social pressure and food safety is not big concern. Mothers' good knowledge on nutrition is not in line with their less nutritious consumption due to children perceived as the center of consumption. These results lead to an understanding that solving nutrition problems depends on community's situation.

KEYWORDS: *Food Style, Feeding Practice, Urban-Semi Urban*

INTRODUCTION

Currently, Indonesia is experiencing the double burden of under-nutrition and over-nutrition. Result from the 2010 Basic Health Research (1) shows the percentage of Low Birth Weight in Indonesia was 8.8%, and among under-five children the prevalence of stunting was 35.6%, wasting was 13.3%, underweight was 17.9%, and overweight was 12.2%.

Nutritional problems are originated from the inability to access food, either because of availability issues at the local level, poverty, education and knowledge of food and nutrition, as well as people's behavior (2). Eating habit has been formed since fetus until the age of five. In this period, attitudes, habits and behavior patterns are developed and play determining role for subsequent individual development (3). Eating behavior of pregnant women and under-five children are affected by food availability, attitudes towards food, portion size, several cultural values, parents' beliefs and practices, feeding time, and feeding method. A family and social environment provide considerable influence on children's attitudes towards food (4). Women's position within the family also determines child's growth and development as it concerned with the allocation of funds and time (3). Mother's feeding practice was influential in determining the pattern of food

consumption in the family. Other determinants are socioeconomic factors, mother's education, mother's occupation, family income, family size, knowledge on food and nutrition, food preparation and serving, information exposure, food prices, and appetite (5).

Thus, it is interesting to understand the pattern of food consumption practice, especially pregnant women and children under five years, among poor families in urban and semi-urban areas in Indonesia.

MATERIALS AND METHODS

The study applied a qualitative approach (Rapid Assessment Procedure-RAP). Data were collected through In-depth interviews with health providers (12), grandmothers (3), cadres (3), food vendors (3) traditional birth attendant (3); and Focus Group Discussion with pregnant mother (3 groups @ 8 persons), mother who has infant (3 groups @ 8 persons) and mother who has child 1-6 years old (3 groups @ 8 persons). FGD was conducted in one of mother houses facilitated by moderator and helped by note taker and all information recorded with tape recorder. East and West Jakarta as representative of urban and Bantul as representative semi-urban were selected as study areas. The study was conducted for 3 months (November 2011 – January 2012). Informants were selected using purposive sampling. All qualitative data were recorded and transferred into transcripts. Data cleaning and processing was using *NVivo software version 2.0*. Subsequently, the data were analyzed using theme analysis.

RESULTS

Food Consumption Pattern among Pregnant Women

Mother's good knowledge on nutrition is not always in line with their practices. In Urban and Semi Urban areas, pregnancy is still perceived as common condition so that pregnant mothers paid more attention in giving nutritious food, such as milk, to their children, rather than fulfilling their own needs. Furthermore, there are food beliefs among pregnant women. 'Cold' foods, such as cucumber, are believed to decrease thirst. Pregnant women are also prohibited to drink iced water, which is perceived as causing large babies, and herbal medicine. However, some of them still consume a small portions despite the disadvantages of those prohibited food / beverages they aware of.

Infant Feeding Pattern (Aged 0-12 months)

Mothers' knowledge of exclusive breast feeding in Semi Urban areas is better than in Urban areas. Unfortunately, inspite of having good understanding about exclusive breastfeeding and its benefits for babies, mothers do not exclusively breastfed their babies and add prelacteal feeding instead, such as porridge, mashed ("scraped") banana, tea, mineral water, and formula milk. Parents or in-laws, which are considered as having more experience, significantly influence mothers to give prelacteal feeding.

There are several factors Urban and Semi Urban mothers have in common and render them to give prelacteal feeding. Breast milk, for its liquid nature, is perceived as insufficient to satisfy babies. Others are retained nipple which prevents breast milk from flowing optimally, working mothers, influence from parents/in-laws, concern on changes on breast shape, and inability to provide breast milk continuously due to tiredness. Most mothers think that formula milk will make babies healthier, gain better brain development, bone growth, and intelligence.

In Urban area, the complementary feeding firstly given are usually instant porridge, softened biscuits, scrapped banana, papaya juice, or squeezed orange juice. Mothers started to give complementary feeding since their baby was 2 months old. Entering the age of 3 months, they started giving home-cooked meals in the form of pounded food, a mix of rice and vegetables (carrots, potatoes, spinach), and blended beef/chicken and a bit of cheese. At the age of 9 months, the baby is given steamed rice (mixed with vegetables such as carrots, potatoes, broccoli; chicken liver, chicken claw and fish) cooked not spicy and used a slight of MSG.

The provision of complementary feeding in Semi Urban area is relatively later than in

Urban areas. Semi Urban mothers begin to give complementary feeding when the baby is 4 or 5 months. The menu is relatively similar to Urban areas. Uniquely, Semi Urban mothers also adds 1 teaspoon of coffee is for it is believed as medication and may strengthen babies' stomach.

Feeding Pattern among Children 2—5 Years Old

In urban and semi-urban areas, differences in diet began to apparent entering the age of 2 years when children begin to consume similar food as adult, except spicy or strong-tasted food like the ones using coconut oil. However, few have already begun to introduce such food to their young child.

The findings show that parents in urban is less able in directing their children in terms of food consumption. Children have more freedom to decide when, what sort, and the amount of food they want to eat. Most parents feel pressured of being reputed as stingy by neighbors if they fail to fulfill their children's want. Mothers easily allow their children to buy food outside home for other reasons, such as no kitchen due to small-sized house and mothers' reluctance to cook. Food vendors in the Urban areas are numerous and varied. Some women in the Urban mentioned that they usually buy side dishes from a stall near home, and prepare the rice themselves.

Paradoxically, Semi Urban mothers usually cook at home and only occasionally buy food from outside home because they consider cooking more economical, reduces the use of flavorings and preservatives, could control the flavor and food hygiene, lack of access to food vendors, and availability of kitchen at home.

Snacking is common among children, particularly in urban areas, ranging from home-production, manufactured food, and main meal. As explained above, parents of under-five children are less likely to control over children craving for snack. Spending on snacks ranges from Rp 5.000 to Rp 20.000 per day, and less in Semi Urban areas.

DISCUSSION

Good knowledge of Urban and Semi Urban mothers is inconsistently with their practice, especially on exclusive breastfeeding which is performed only by a small fraction of mothers. Babies are breastfed on demand and supplemented by prelacteal feeding, primarily based on the advice from the mothers/in-law. Breastmilk is considered insufficient so that they need to add solid food, beverages, or formula milk to satisfy the babies. These findings are consistent with that stated 34% of women give prelacteal food before the baby reached the age of 6 months due to fears of inadequacy of nutritional need from breast milk only (6).

"Snack" is food or beverage consumed apart from meal (breakfast, lunch and dinner) times (7). For under-five children, snack may act as nutritional supplement given nutritional needs are increased among this group and, hence, the selection of good quality snack becomes very important. Unfortunately, snacking behavior often does not take into account the nutrient contents and safety. Snacks are usually rich in fat and sugar, contain harmful substances (textile dyes, prohibited synthetic sweeteners, additives such as borax and formalin, multiple-used cooking oil), as well as lack of hygiene during processing (8). Although having less quality than home-made food, many people still prefer snacks or street food due to practicality, low price, more variation, etc.

Urban parents are less able in directing their child toward good consumption practice. Similar finding that Latino children often determine 'what' food served and 'when' (9). This pattern may render Urban children into poor diet and unmet need of nutritional requirements thereby, not to mention the poor hygiene and unsafe substances. When In the long term, such condition could impair growth and development. Unlike in Urban area, Semi Urban mothers, or grandmothers, are still trying to provide home-cooked food, thus ensuring better quality of their children's nutritional intake.

CONCLUSION

Results of this study lead to conclusion that children's feeding pattern is highly influenced by social and economic factors. Apparent gap exists between mothers' feeding practice and

their knowledge on nutrition for pregnant women. Children become the center of consumption and their satisfaction becomes mothers' highest concern. Healthy foods like milk are more prioritized for children than for pregnant women. Snacking has becomes part of lifestyle, influenced by the high access to various food vendors with affordable price. Many mothers prefer to buy food outside home due to practicality, low price, less time consuming, lack of kitchen facilities at home, and lack of cooking skill. This study suggest the need to take into account the aforementioned external factors in designing nutrition program in line with community's situation, in terms of social, economic and culture.

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Mapping and Risk Factors Analysis of Toxoplasmosis at Yogyakarta Special Province and Bali through EcoHealth Approaches

Wayan T. Artama[1] Sujono[2] Adhiheru Sutomo[3] DyahAyu Widiasih[4] Tjut Sugandawati Djohan[6]
Pande Made Kutanegara[7] DewaAyu Sri Laksmi[5] Fihiruddin[8]

[1] *Department of Biochemistry Faculty of Veterinary Medicine, Universitas Gadjah Mada (UGM)*

[2] *Health Polytechnic, Yogyakarta*

[3] *Department of Public Health Faculty of Medicine, UGM*

[4] *Department of Public Health Faculty of Veterinary Medicine, UGM*

[5] *Department of Parasitology University of Udayana*

[6] *Faculty of Biology, UGM*

[7] *Department of Anthropology Faculty of Social Culture, UGM*

[8] *Health Polytechnic Mataram*

ABSTRACT Toxoplasmosis is a crucial public health problem because of its high economic and social impact in relation to cost of patient care, born defect such as hydrocephalus, mental retardation, retinochoriditis, and blindness in children. Environmental risk factors that likely increase the spread of toxoplasmosis in the Special Province of Yogyakarta and Bali are the presence of definitive host in the population, altitude, land surface temperature and climate condition that enable the development of oocyist, high prevalence of toxoplasmosis in goats and cows (49% and 21%), eating habits and non chlorinated water. The aims of this research is to study serological prevalence of toxoplasmosis in the population and get an overview of association between risk factors and toxoplasmosis infection, comprising gender, age, geography, contact with cats, eating habits, occupation related to contact with raw meat, water used to cook and activities related to contact with soil trough EcoHealth approaches. The amount of 1050 serum samples were collected using double cluster design from Special Province of Yogyakarta and 960 serum sample from Bali. Data of risk factors were obtained through interview with respondents or parents of respondents. Anti toxoplasmosis IgG and IgM detected with ELISA method using recombinant protein of GRA-1 tachizoites of local isolate *Toxoplasma gondii* as an antigen. Positive and negative control used human serum that had been positively or negatively diagnosed at Dr. Sardjito, Bethesda and Panti Rapih Hospital Yogyakarta. Risk factors were analyzed their significance and odds ratio at confidence interval 95%, and mapping of serological prevalence with Geographical Information System. Serological prevalence of toxoplasmosis at the Special Province of Yogyakarta was 61, 5%. Highest at District of Kulon Progo (78.6%), later at District of Sleman (72.4%), at the city of Yogyakarta (69.5%), at District of Bantul (57,6) and at District of Gunung Kidul (29.5%) and Bali (39%). Risk factors significantly associated with serological prevalence of toxoplasmosis at the Yogyakarta Special Province examination were gender, ad for residence plain (geography), contact with cat,

undercooked goat meat consumption, raw vegetable consumption at food stalls, occupation related to contact with raw meat, and occupation/activities related to contact with soil. Age, undercooked chicken and beef meat consumption, raw vegetable consumption at home and water used to cook were insignificantly associated with serological prevalence of toxoplasmosis.

KEYWORDS: *Toxoplasmosis, IgM, IgG, GRA-1 recombinant protein, ELISA, EcoHealth*

INTRODUCTION

Toxoplasmosis is an important zoonotic diseases caused by protozoan Toxoplasma gondii that is distributed world-wide. Warm-blooded animals including humans and other mammals, are its intermediate hosts, while cats and other various types of Felidae as definitive hosts. The prevalence of toxoplasmosis in goats and cattle in Yogyakarta was 78% and 21% (1-5). Toxoplasmosis in humans and animals is often show subclinical symptoms or no symptoms, so a person or animal unwittingly suffering from toxoplasmosis. Humans can be infected by *T. gondii* through food, undercooked meat, and vegetables contaminated with oocysts or by congenital transmission from pregnant mother to fetus. Transmission of zoonotic diseases besides direct contact with animals could be caused by ecological factors, such as weather changes, climate, and environment. The environment are includes everything outside the relevant of organisms such as sunlight, temperature, humidity, topology, vegetation, rainfall and competitors (12, 13). Cats play a role in spreading toxoplasmosis in animal, wildlife and humans. The changes in environmental conditions including temperature, topography region, the presence of animals farm and distribution of the population can affect against prevalence of toxoplasmosis, thus it's necessary to study the prevalence of toxoplasmosis in Yogyakarta through Ecohealth approaches. Using GIS to provide spatial data that describe the distribution or pattern of spread of a disease and to know prevalence and populations at risk for a disease that was observed based on Ecohealth concept.

MATERIALS AND METHODS

This study is an analytical survey with Cross-Sectional Design. Blood sample from human were obtained from each district and total samples from Special Province of Yogyakarta were 1050 respondents and Bali 960 samples. The sample was collected from October until December 2012, and then sera were kept frozen at -20°C. The choice of respondents performed with 2 cluster sample design stage, that is probability proportionate to size (PPS) and simple random sampling. The objectives of this research were indentified sera prevalence of toxoplasmosis in human using GRA recombinant protein as antigen on ELISA test and the relation of animals, environment and risk factors to the diseases and also mapping of the diseases in Yogyakarta and Bali region.

RESULTS AND DISCUSSION

The prevalence of toxoplasmosis by ELISA test using recombinant protein GRA-1 from *T. gondii* local isolate shown that anti- IgM and IgG Toxoplasma positive in DIY was 61.7%, Kulon Progo belong to highest in the District is 78.6%, Sleman 72.4%, city Yogyakarta 69.5%, Bantul 57.6% and the lowest in the Gunung Kidul that is 29.5%. Toxoplasma gondii can infect the intermediate host in a very wide range. Toxoplasmosis in cats, livestock and wild animals are a source of transmission to humans. Differences in prevalence of this disease in each sub-district at Sleman (Figure 1) due to differences in behavior, environmental conditions and demographics of the community in each sub district. The risk factors that greatly influence the prevalence of toxoplasmosis incidence in an area were
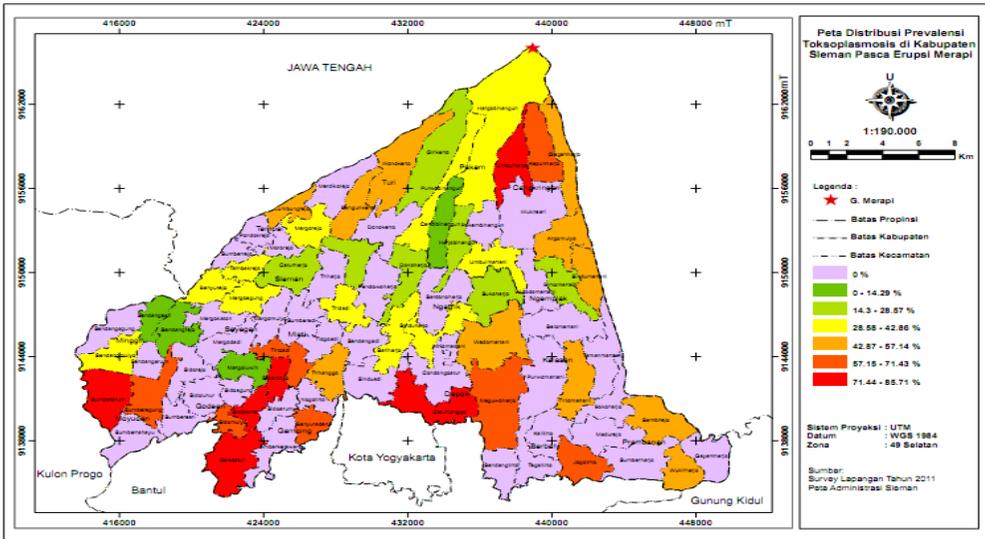
Figure 1: Sera -prevalence distribution of toxoplasmosis in Sleman district based on each of clusters.
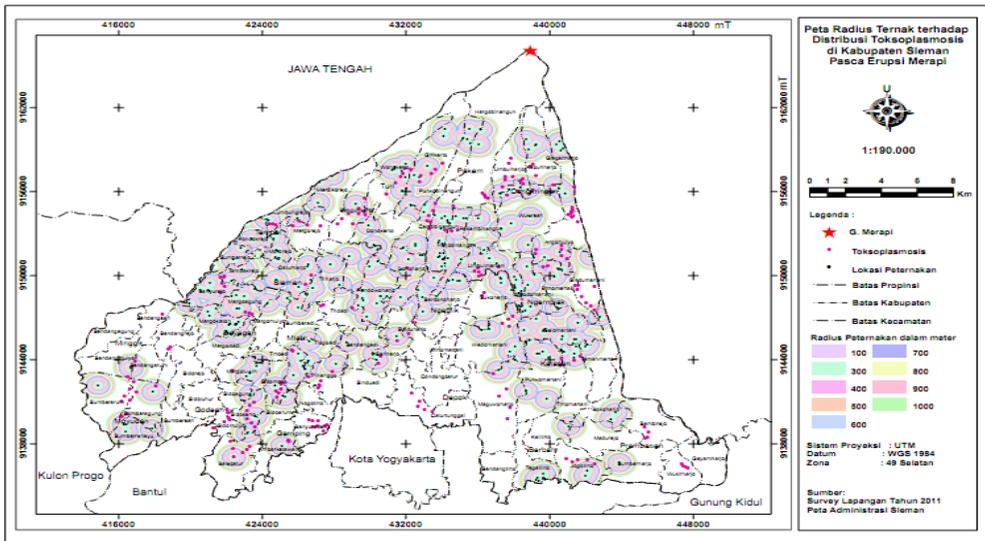
Figure 2: Distribution of toxoplasmosis in around the cattle barns in Sleman District.

behavioral, geographic environment and the presence of cats.

Differences the prevalence of toxoplasmosis is expected due to different of conditions in each regions, climate, farming methods, customs, meat consumption habits, andcontactwithcats (9, 10). The eruption of Merapi was changes of environmental conditions that will cause any changes in the ecology of species that exist in it, including the existing vegetation within the ecology. Vegetation is very important for breeding of

annelids and arthropod like flies and cockroaches, which acts as a vector to move oocysts in the environment to livestock or humans. Meat of animals such as goats, sheep, pigs, cattle, poultry and other livestock plays an important role as a source transmission of *T. gondii* to human. Intermediate host like animals and environment is factor that could break the chain of disease caused by *T. gondii*. The reduction population of cats that were around the area of eruption influence against the number of oocysts that contaminate the

environment (4, 9). Sample were taken from 25 m, 50 m, 100 m, and above 200 m of the rivers was showed percentage positive are 59, 2 %, 54, 8 %, 46, 1 %, and 66, 4 % respectively. The transmission of the disease depends on the hydrologic characteristics of the river because the river characteristics related to water conditions in the cross section of the river. Based on indications of proximity to cross the river, toxoplasmosis is found around the river. Oocyst contaminate environment through surface water as river following the flow of rain water. Residents who live around the river was possibility using river water for necessities such as watering the yard or other need, so if there were oocysts in these watersheds, it will potentially transmit toxoplasmosis to humans or pets around human settlements. Oocysts were very resistant in an aqueous environment and many disinfectant including chemicals like clorine (7). Inhabitant settlement close to the cattle barns found positive cases of toxoplasmosis. Residential within 100 m, 200 m, 300 m, and above 300 m of the barn was shown that percentage of toxoplasmosis positive are 34,7 %, 26,9 %, 37,5 %, and 76,2 % respectively. Toxoplasmosis cases in the residents who live around the cattle shed is due to contamination of food or drinking water by oocysts of *T. gondii that* derived from cattle barns carried by flies or cockroaches. Cats often looking for food around the barns so it will defecation in the corral. Oocysts that contaminate the environment can be distributed through the air, surface water, rain water, and crops, as it also can spread through soil worms and compost. The results of IgM and IgG anti toxoplasma test are based on several of temperature showed significant results among several soil surface temperatures in the value *(p-value 0.023)*. Weather and geographic location differences greatly affect to spread of *T. gondii* in the environment. *Toxoplama gondii* does not undergo sporulation, survive and become infectious without the influence of climatic conditions. Yogyakarta is wet tropical climate with an average temperature of 26 - 27°C and the humidity is 70%-95% (in 2010).

Temperature and humidity in the area are an ideal climate conditions for sporulation process of *T. gondii* oocysts. The results of satellite imagery, ground surface temperature in Yogyakarta are ranged between 23-36°C. The sporulation process of *T. gondii* oocyst is occurs at a temperature of 20-28°C. The sporulation process usually ranges from 1 to 5 days depending on humidity and temperature of the surrounding environment. The oocysts form had done sporulation are very resistant in the outside environment and infective for 1 year or more at a temperature of 24°C, but if it's at a temperature of 30°C is only lasting for 20 days and at 50°C or more then will quickly die (12, 13). Results of toxoplasmosis test at various temperatures was show that temperatures at 25-30°C (54.4%), 26-30°C (66.4%), and a temperature above 30°C (48%). These results were indicating that the environment with a temperature above 30°C led to oocyst sporulation process so will be longer, so that influences against the prevalence of toxoplasmosis in cattle or humans. Based on the altitude where the prevalence of toxoplasmosis showed different results, that is 0-150 meters (66.3 %), 150-500 meters (61.9%), and a up of 500 meters (41.1%). Statistic test with a Chi square test showed significant values (p-value 0.001) among of it. The prevalence of toxoplasmosis were based on altitude in the area can be influenced by several factors including the presence of oocysts in the environment and public behavior (Fig. 2).

The number of oocysts at an altitude of more than 500 meters less than the height 0-150 meters due to number of oocysts in each height is influenced by the environmental damage caused by the eruption of Merapi. Besides that the oocysts in height area can be carried by rain water through the river to areas of lower altitude, so the number of oocysts in these areas will increase. At an altitude of 500 meters above, the area which showed a high prevalence of toxoplasmosis are Cangkringan (60%), where the area is most severely affected by the eruption of Merapi. High prevalence of toxoplasmosis in this area is probably due to poor hygiene in the

consumption of food or drink with appears new cases of toxoplasmosis are shown by positive IgM (16%). Source of transmission of toxoplasmosis is comes from vegetables or drinking water, where the vegetables are not washed clean because limited of water. Decline in public health conditions also resulted in the emergence of re-infection cases of toxoplasmosis, where the results of IgG and IgM positive test is high (14%). Result of Multivariate test by Binary Logistic Regression is shows that Odds Ratio the population of cats in the settlements the most impact when compared with other environmental factors is 1.729. The presence of cats is found in areas close to urban areas. In urban areas are usually very dense population and there are many sources of food for cats like waste place is a source of contamination for humans or animals (4).

CONCLUSION

The prevalence of toxoplasmosis in Yogyakarta after eruption of Merapi was 58 % and the distance of rivers and cattle pens had affects against toxoplasmosis. There are differences sera-prevalence of toxoplasmosis were based on altitude at 0-500 and above 500 meter, soil surface temperature between 20-30°C and above 30°C and presents of cats inhuman settlements.

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Increasing Farmers' Knowledge of Beef Cattle Manure Fermentation and Zoonotic Diseases Based on Eco-Health Principles

Aris Purwantoro[1,3,*] Adi Heru Husodo[2,3] Drajat Purbadi[4]

[1] Department of Biochemistry, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia
[2] Department of Public Health, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia
[3] EcoHealth Resource Center, Universitas Gadjah Mada (EHRC-UGM),Yogyakarta, Indonesia
[4] Department of Marine, Fisheries, and Livestock Services; Kulon Progo Government, Yogyakarta Special Region, Indonesia
*Corresponding author: Email: a_purwanto_fkh@ugm.ac.id

ABSTRACT Beef cattle is the most popular large ruminant to be raised in Indonesia and the population reaches 12.76 million heads. However, the manure just abandoned beside or inside barn without any treatment due to limited farmers' experience. Furthermore, the manure could spread zoonotic diseases and contaminate surrounding environment. In order to solve the problems above, the manure has to be processed to kill pathogenic microbes, either via aerobic or anaerobic fermentation. Two hundred seventy farmers were participated in this program to evaluate their empowerment on the manure fermentation based on EcoHealth principles. They were chosen randomly from nine sub-villages of Donomulyo and Wijimulyo Villages at Kulon Progo Regency. The farmers were asked using questionnaire during pre- and post-treatment of above program, then the data were analyzed statistically. The program conducted by EcoHealth Resource Center, Universitas Gadjah Mada (EHRC-UGM) and led by the university lecturers and assisted by the students through community services. The result showed that there was a significant difference ($P<0.05$) between pre- and post-treatment. Thus, empowering farmers could increase their knowledge of beef cattle manure fermentation and zoonotic diseases based on eco-health principles.

KEYWORDS: *farmers' knowledge, beef cattle, manure fermentation, zoonotic diseases.*

INTRODUCTION

Indonesia is a developing country locates in tropical zone which has mega biodiversity of flora and fauna. This natural resources support agricultural development. For example, the current population of beef cattle reaches 12.76 million heads. It becomes the most popular large ruminant to be raised by farmers compared with dairy cow and buffalo. The livestock has many functions, such as: meat producer, plowing power, life deposit for instant cash (1).

Current human population in Indonesia is more than 237.64 millions. Their occupation mainly is in agricultural sector (up to 44.92%). The farmers mostly live in rural areas where unoccupied lands still exist. Unfortunately, they have low education and skill (2).

The above conditions trigger some problems, for instance: beef manure just abandoned beside or inside barn. The farmers do not treat it due to limited experience. Furthermore, they do not realize that the

manure could spread zoonotic diseases and contaminate surrounding environment (3, 4).

The manure fermentation could reduce zoonotic diseases spreading. For instance, the fermentation could killed avian influenza virus (H6N2) at day-6 at thermophilic temperature compared to ambient-composting at day-21 (5). The thermophilic method is usually more effective to destroy pathogen than mesophilic digestion of manure (6). Furthermore, the thermophilic anaerobic treatment of manure can be effective for inactivation of vegetative-bacteria, viruses with moderate resistance, and infectious-stages of parasites (7).

In order to solve the problem above, the farmers' knowledge on manure fermentation and zoonotic diseases has to be increased by related authorities for sustainable agriculture.

MATERIALS AND METHODS

The program to solve beef cattle manure above was created together among EHRC-UGM, farmer-groups, and local-government. The program was designed to solve the problem comprehensively using entire available resources.

The study was conducted at nine sub-villages (Bandung, Wareng, Lengkong, Donomerto, Karangwetan, Penjalin, Cepitan, Temanggal, Tegalsari), Donomulyo and WijimulyoVillages, KulonProgo Regency, Yogyakarta Special Region, Indonesia. The study was implemented from October 2011 to September 2012 which devided into three cohorts (i.e. October-December 2011, March-April 2012, and July-August 2012).

The farmers from above locations were practiced to treat the beef cattle manure using both aerobic and anaerobic fermentation based on eco-health principles. The program was conducted by EHRC-UGM and trained by the lecturers and students through community services.

The aerobic fermentation was performed in several pilot-plants at each sub-village. The manure by mixed with water until 70% of water-content in order to reduce ammonia and nitrous-oxide emissions by 30-70% (8). The mixture was layered every 20 cm thick until 100 cm pile then sprayed by thermophilic

starter (9) which resulted carbon losses until 26.4% (10). The farmers covered compost-pile with plastic-cover and aerated by perforating rather than turning the pile to in order to reduce larger changes of the physical properties and weed germination (11, 12) and minimize green houses gas (GHG) emission (13, 14). By using the compost, the farmers could increase their crop production and soil properties (15).

The anaerobic fermentation was performed using a 12 meter-cubic fermentation-tank. The EHRC-UGM only installed a biogas-digester due to very expensive materials. The digester could produce biogas until 60% purity of methane and improve the quality of livestock waste (16). The biogas production as renewable-energy was not only strip out problematic odor compared to composting method, but also could be utilized it for cooking, lighting, and heating (17, 18).

Two hundred seventy farmers were chosen randomly to participate in this program. They were asked using questionnaire during pre- and post-treatment. The data were tabulated and analyzed with paired t-test using statistical software (SPSS version 16.0, SPSS Inc., USA).

RESULTS

The training of beef cattle manure fermentation for farmers divided into two steps. The first step was a training-of-trainers (TOT) which led by UGM lecturers for village-leaders and students. The second step, the TOT-participants trained the rest of farmers and villagers which supervised by the students. The training carried out twice in each sub-village with different cohort of community services.

At the initial stage of those trainings, the trainers make farmers aware of zoonotic diseases from abandoned manure and realize of advantages from fermented manure. The next stage, the trainers assisted participants to ferment manure aerobically using the pilot-digester As a result, the farmers have knowledge and skill for utilizing abandoned

manure to produce compost and biogas by their own.

The students asked farmers using questionnaire before (pre) and after (post) the treatment above. The questions asked to the farmers regarding knowledge of zoonotic diseases, manure processing, aerobic and anaerobic fermentation. Data of above answers are presented at the table below.

Table 1: Total farmers who have knowledgeof zoonotic diseases and beef cattlemanure fermentation.

Parameter	Treatment	
	Pre	Post
1. Zoonotic diseases	246	263
2. Manure processing	191	213
3. Aerobic fermentation	209	239
4. Anaerobic fermentation	162	201

Note: n = 270

The statistical result as can be seen at above table that shows a significant different (P<0.05) of the farmers' knowledge regarding zoonotic diseases, beef cattle manure processing, aerobic and anaerobic fermentation compared pre- with post-treatment.

DISCUSSION

The program as can be seen from the table above increased farmers' knowledge of zoonotic disease and manure processing from 246 to 263 and from 191 to 213, respectively. The number of farmers who have knowledge of aerobic and anaerobic fermentations also rose from 209 to 239 and from 162 to 201, respectively. Thus, there was a significant difference (P<0.05) of the farmers' knowledge compared pre- with post-treatment.

The success of program above for empowering farmers to ferment beef cattle manure was affected by some reasons as follows. The EHRC-UGM selected two-steps strategy of the training during farmers' leisure time. The village-leaders were actively involved as participants at the first-training then as trainers for the second-training. Thus, they have important role to empower both farmers and community members.

The eco-health principles were applied at the program with some examples as follows. The program supported the farmers to understand the complex relationship among people, health, and surrounding ecosystem in the context of social-ecological systems (as *system-thinking* aspect). The program based on the UGM lecturers' research-results and experiences (as *knowledge to action* aspect). Both lecturers and students come from different faculties, so they implemented the program through integrative approaches (as *trans-disciplinary* aspect). The farmers' leaders became cadres of the program (as *sustainability* aspect). The farmers also supplied their local-resources and labor (as *participation* aspect). The program was followed by all gender and social status, such as: male and female, old and young, members and farmer-leaders (as *equity* aspect)(19, 20).

CONCLUSION

It can be concluded from the program above that empowering farmers could increase their knowledge of beef cattle manure fermentation and zoonotic diseases based on eco-health principles.

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Ten-year Epidemiology of Human Rabies in Thailand, B.E. 2546 – 2555 (2003 – 2012 AD)

Teerasak Chuxnum[1,2*] Soawapak Hinjoy[1] PrawitChoomkasien[1] Sompoch Ratioran[2]
[1] *Bureau of Epidemiology, Ministry of Public Health*
[2] *School of Health Science, Sukhothai Thammathirat Open University*
Corresponding author; Email: tchuxnum@yahoo.com

ABSTRACT   Rabies has been a notifiable disease under public health surveillance systems in Thailand. The study of human rabies epidemiologic patterns of will lead to the control strategies to eliminate rabies. An assessment of the human rabies was derived from the routine infectious disease surveillance system and all individual case investigations. The human rabies from B.E. 2546-2555 was fluctuately decreased. There were 166 cumulative cases distributed in all four regions. The highest cases were found in the Central and South regions. Bangkok, Kanchanaburi and Songkhla were the highest ranks endemic area. The cases were found throughout the year. Dogs were the main cause of the deaths. Of the dogs, 43% were below 3 months and 18% were more than 1 year old. Among these dogs, 66% of the dogs with owners had not received rabies vaccination. Among the cases there were 37% deep wound bites, 19% lacerated wounds and 16% abrasion/scratch wounds. The most common parts of bodies affected were finger, wrist and hand (33%). Most of the human rabies cases had not received rabies vaccine after the animal contact or bite. The prevention and control strategies should integrate the animal surveillance and animal bite surveillance.

KEYWORDS: *Human, Rabies, Epidemiology, Thailand*

INTRODUCTION

One of the oldest recognized zoonotic diseases, Rabies is still an important infectious disease listed by the World Health Organization. In Thailand, it has been a notifiable disease within the public health surveillance systems since B.E. 2524 under the Bureau of Epidemiology, Ministry of Public Health (1). It is endemic in all regions of Thailand. This Rabies surveillance is a tool to measure disease burden in order to prevent and control the disease in elimination period. Human rabies positive cases are diagnosed by history and symptoms, particularly hydrophobia. Confirmed cases were verified by laboratory testing (2). Although the numbers of human rabies cases have been fluctuately decreasing over the past ten years, there have still been the cases in the same areas which indicated the source of infection in those areas. However, the epidemiological situation has started to change. The objective of this study was to summarize the epidemiologic features of human rabies in Thailand from B.E. 2546-2555. These results will be used to establish strategies and pinpoint current problems to eliminate human rabies cases in Thailand by B.E. 2563 (2020 AD).

MATERIALS AND METHODS

Assessment of human rabies was based on information derived from the routine infectious disease surveillance system. The human rabies case definition for surveillance is included (2):

Clinical criteria

A patient with fever, headache, pain or itching at the site of a mammal bite; spasm of swallowing with a history of a mammal bite, scratch or contact, and with one of the following symptoms: hydrophobia, aerophobia or photophobia, myo-edema, paresis or paralysis.

Laboratory criteria:

- Antigen detection by direct fluorescent antibody test (FAT) or
- Virus isolation in a laboratory animal or in cell culture or
- Identification of antibody titer by Mouse Neutralization Test (MNT) or
- Rapid Fluorescent Focus Inhibition Test (RFFIT), or
- Detection of viral DNA by molecular methods

Surveillance data on human rabies, collected by the Bureau of Epidemiology from all Provincial Health Offices, consisted of annual cumulative numbers from B.E. 2546-2555 and all individual case investigation forms which were received. These were analyzed for distribution and risk factors associated with rabies in humans.

RESULTS

A rapid decrease was seen from B.E. 2541-2545 with 57, 68, 50, 37 and 30 cases per year respectively. After that, from B.E. 2546-2545, the cases fluctuately decreased to 21, 19, 20, 26 and 20 cases per year respectively. In B.E. 2551, 2554 and 2555, the cases were below 10 cases per year. Over the recent ten years, there were mean = 17 cases and standard deviation = 7. The mortality rate each year has shown in Table 1.

There were 166 cumulative cases distributed in all four regions. The highest cases were found in the Central region with 101 deaths in 19 provinces. The other regions, in order of severity showed the following: Southern region, 30 cases in 7 provinces, Northeastern region, 28 cases in 10 provinces, and Northern region, with 7 cases in 3 provinces. The highest mortality rates were found in the Central and Southern regions. The first 3 highest ranking groups were found

in Bangkok with 25 cases, Kanchanaburi with 14 cases and Songkhla with 12 cases.

Table 1: Human rabies cases from B.E. 2546 – 2555 in Thailand.

Year (B.E.)	Cases	Mortality rate (per 100,000 populations)
2546	21	0.03
2547	19	0.03
2548	20	0.03
2549	26	0.04
2550	20	0.03
2551	8	0.01
2552	24	0.04
2553	15	0.02
2554	8	0.01
2555	5	0.01

Of the 166 cases, 71% were male and 29% were female, but these proportions varied each year. The mortality rate also varied in age groups. The cases were found throughout the year with the animal contact or bite history varying during the time period. Dogs were the main cause of the deaths for rabies, responsible for 86% of human deaths. Cats were responsible for 4% of human deaths.

Of the dogs, 43% were younger than 3 months, 8% were 3-6 months old, 3% were from 6 months of age – 1 year of age, and 18% were more than 1 year old. Among these dogs, 66% of the dogs with owners had not received Rabies vaccination each year.

Among the cases there were 37% deep wound bites, 19% lacerated wounds and 16% abrasion/scratch wounds. The most common parts of bodies affected were fingers, wrists and hands (33%), toes, ankles and feet (13%), arms, body, chest and neck (11%), calves, shins, knees, thighs and hips (13%) and chin, mouth, nose and cheek (8%). Most of the

Table 2: Human rabies in Thailand by region and province from B.E. 2546 – 2555.

Reporting areas	Cases	Reporting areas	Cases	Reporting areas	Cases
Central Region	101	Northeastern Region	28	Southern Region	30
Bangkok	25	Surin	6	Songkhla	12
Kanchnaburi	14	Ubonratchathani	5	Nakhonsithammarat	8
Rayong	10	Buriram	4	Phatthalung	4
Chonburi	8	Sisaket	4	Suratthani	3
Ratchaburi	7	Mukdahan	3	Krabi	1
Samutprakan	7	Loei	2	Ranong	1
Suphanburi	5	Chaiyaphum	1	Yala	1
Chantaburi	4	Nakhonratchasima	1	Northern Region	7
Prachinburi	3	Nongbualamphu	1	Chiangmai	3
Sakaeo	3	Sakonnakhon	1	Tak	3
Angthong	2			Phichit	1
Ayutthaya	2				
Nakhonpathom	2				
Pathumthani	2				
Samutsakhon	2				
Saraburi	2				
Nonthaburi	1				
Prachuapkhirikhan	1				
Samutsongkhram	1				

human rabies cases did not receive post-exposure Rabies vaccine after the animal contact or bite. There were 11 cases who received the post-exposure Rabies vaccine; six of them had delayed vaccination, three of them did not complete the dose schedule, one of them was infected with HIV and the other had repeated HRIG after the first vaccination.
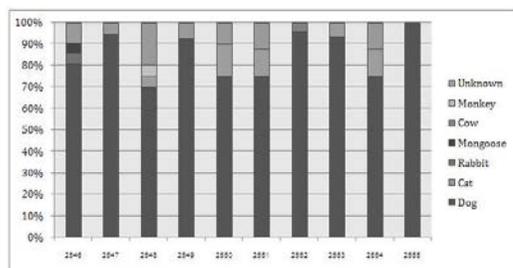
Figure 1: Percentage of animals that cause human rabies by year.

The shortest incubation period was 4 days, the longest incubation period was 1,153 days (Median = 64 days). The incubation period depends on parts of the body affected and
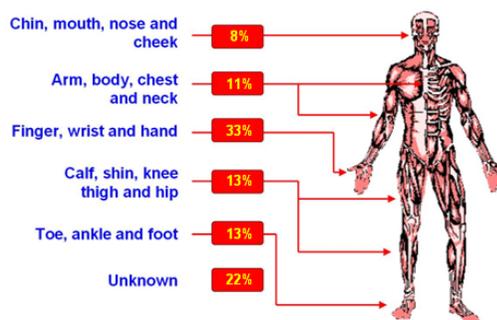
Figure 2: The parts of the body affected.

severity of wound. The stages of clinical sign period were longest in 26 days and shortest within 1 day (Median = 5 days).

DISCUSSION

Although the human rabies cases have decreased from the past, the epidemiological situation has changed. Ancient Thai beliefs suggest that rabies can be found not only in the summer, but throughout the year. Gender and age groups at risk vary each year. Dogs were the main rabies reservoir and cause of the cases as in the past. However, the age of

dogs below 3 months was higher compared with the previous rates, during which the dogs were more than one year of age (3). Also, in this recent study, unvaccinated dogs which had owners were the major cause of the cases in comparison to the past in which stray dogs were the primary source (3). The regions that should be focused on for prevention and control strategies were the Central and Southern regions, especially the provinces which had the highest number of cases, or with continuing incident cases each year. In order to prevention and control strategies should integrate the animal surveillance and animal bite surveillance.

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Community-based Model for Rabies Control in Bali: An EcoHealth Approach

Andri Jatikusumah[1,*] Ridvana Dwibawa Darmawan[1] Maria Digna Winda Widyastuti[1] Sunandar[1]
Edi Basuno[2] Anak Agung Gde Putra[3] Iwan Willyanto[4] Soelih Estoepangestie[5] Jeffrey Gilbert[6]
[1] *Center for Indonesian Veterinary Analytical Studies, Indonesia*
[2] *The Center for Agriculture Socio Economics and Policy Studies, Ministry of Agriculture, Indonesia*
[3] *Disease Investigation Centre Denpasar, Indonesia*
[4] *InI Veterinary Service, Indonesia*
[5] *Airlangga University, Indonesia*
[6] *International Livestock Research Institute, Kenya*
Corresponding author; Email: andro_fkh@yahoo.com

ABSTRACT In 2008, Bali was struck by rabies and since then rabies had spread to 273 of 723 villages in Bali and the number of human fatalities had reached 145. The concerted effort of government and stakeholders to combat this disease is progressing well. At present, the government's control program consists of mass vaccination, selected targeted culling, and education with a goal to eradicate rabies from the island by 2015. The additional effort on the establishment of participatory rabies prevention, control and surveillance system within the local community level is needed to improve the ongoing and future rabies control efforts. As part of a project "Ecohealth Approaches for Optimizing the Rabies Control Program in Bali", organized by CIVAS, with support from the IDRC and led by ILRI, within the project, we developed and piloted a community-based model for sustainable rabies control driven by community involvement in two villages in Karangasem and Gianyar districts in Bali. Promising results from pilot villages model indicates high participation of the community (formed 82 cadres in Gianyar and Karangasem) on the public awareness, early reporting, early response and early detection on bitten cases and supporting on government program particularly on the mass vaccination by registration data of the dog within community to achieved high coverage on vaccination (>70%). This efforts suggests that involving community members more actively in villages through the institutionalization of 'rabies village working groups' could substantially improve surveillance activities with possible effects on improving dog management and vaccination coverage. With little direct financial input such participation creates a sense of community ownership. This approach showed affective on improving early response, early detection and surveillance system that could be used effectively across other districts in Bali. The continued fight against rabies on Bali and elsewhere in the developing world would benefit significantly from engaging communities more actively in control and surveillance efforts.
KEYWORDS: *Community Based Approach, Ecohealth*

INTRODUCTION

Historically, Bali has been free from rabies. In 2008, Bali was struck by rabies and since then rabies had spread to 273 of 723 villages in Bali and the number of human fatalities had reached 145 (1).

Government response in the early days of the outbreak was swift. At present, the government's control program consists of phases of mass vaccination, selected targeted culling, and education with a goal to eradicate rabies from the island by 2015 (2). The first island-wide mass dog vaccination phase started in October 2010 with the third phase wrapping up on January 2013. Based on the demography study within this project estimated high vaccination coverage (>70%) (3).

Despite these efforts, rabies has not been eradicated from Bali. Center for Indonesian Veterinary Analytical Studies (CIVAS), with support from the International Development Research Center (IDRC) and led by International Livestock Research Institute (ILRI), in partnership with livestock and human health officials developed and piloted a community-based model for sustainable rabies control driven by community involvement in two villages in Karangasem and Gianyar districts in Bali, in order to help local governments to optimize rabies control program, support the mass vaccination and surveillance program as a community-based approach to prevent and control rabies within local communities.

MATERIALS AND METHODS

The development of the pilot villages as part of community engagement activities were carried out in the two pilot villages namely Sibetan (villages with human and dog rabies cases) in Karangasem District and Melinggih Kelod (villages with rabies cases in dogs) in Gianyar district. The Activities was done from February 2011 to October 2012. There are two targets in this activities, (1) school community and (2) sub-villages (banjar) community.

We used Ecohealth approach (4) and outcome mapping (5) in this activities. The community participation was developed based on needs, assessment on community experience from the field then basic information that developed into action plans.

The involvement of public participation is carried out through personal and groups approach such as visited formal/informal leaders and religious leaders, social meeting such traditional monthly meeting, farmers meeting, small informal discussion, etc.

In General, the community engagement activities were accomplished in three inters linked-phases.

1. Profiling the villages and stakeholders mapping within the village.

This phase was done by collecting the general information (e.g. geographical information, demography information, social information) and others characteristics (e.g. public education infrastructure, cultural information) of the pilot villages. This information was used as basis information to develop strategy to engage the community of pilot villages and potential stakeholders as boundary partners.

2. Implementation Program Banjar Community

a. Village Animal Health Worker/Cadre development in Banjars (sub-villages) level

The rabies cadres were built around our understanding that a lack of information about rabies in communities is a barrier to rabies control program and that trusted members of communities can be mobilized to share information that will optimize and support the rabies control program.

Members of the cadres selected based on recommendation by village heads and then provided with a 2-day training on rabies in animals and humans, responsible dog ownership, appropriate, rapid response to dog bite cases and reporting system to the local authorities (as part of surveillance system), data collection as part of the dog registration program, and messages to convey to communities as part of public awareness activities. Cadres were organized into groups of men, women, and young people in order to effectively reach different segments of the community.

b. Public awareness activities in Banjar level Communities

We approached the traditional monthly meeting in Banjar level for the fathers group, and for the mother group we approached the traditional monthly meeting and Posyandu (*Pos Pelayanan Terpadu*, a health center in banjar level for maternal and child that formed by Ministry of health) for mothers. Within youngster we approached through youth community (Truna-Truni and Karang Taruna).

We provided general rabies information, bite case management, pet responsible ownership, rapid response on rabies cases, etc. that we delivered through presentation and video and every session followed by discussion.

c. Dog registration in Banjar

The supporting activity in banjar level that implemented by cadres is a dog registration program. Dog registration cards and rabies vaccination cards are given to dog owners by livestock services. Members of the cadres compile information from the cards completed by the dog owners in a log book, which helps monitor the dog population at the banjar-level.

School Community

a. Public Awareness Activities
Public awareness activities to School Communities

CIVAS with officers from District Livestock Services and District Health Services developed informational and awareness materials on rabies and delivered the program in two sessions, in the first session, we provided general information on rabies and how to avoid and respond to dog bite cases. The second session focused on responsible dog ownership, emphasizing the importance of dog registration. Classroom presentations, a film, songs, scenarios, and interactive discussion kept students engaged and reinforced concepts.

We covered similar topics in a seminar tailored to elementary and junior high school teachers. The seminar served to provide teachers with the information and tools for incorporating rabies awareness in their own classrooms and connected them with community leaders involved in rabies control efforts, like livestock and health service officers and village leaders.

3. Monitoring and evaluation in Banjar Communities and Students

Monitoring and evaluation activity was done actively by doing a visit to banjar and cadres. The evaluation activities was done on cadres activities on public awareness, dog registration system, activities on early response, early detection and reporting process to the local authorities. The evaluation was also done to look at changes in the knowledge and behavior of society, which carried out through questionnaires, observation data retrieval behaviors and in-depth interview with results based cadres.

The evaluations on vaccination coverage was done by door to door survey (part of dog demography study) within the villages by asking and check the vaccination status of the dog in each houses after the mass vaccination done.

The increasing knowledge in the school was done by pre and post-test. The post-tests were implemented two times, a week after and 3 months later after all the material was gave to the students.

Indicators

We developed indicators to see the achievement level of two pilot villages. Those indicators are:

1. Improvement the level of knowledge on rabies (general information, bite case management, early response and early detection) within the targets (school communities and banjar communities).
2. High coverage vaccination (as part of the dog registration system and supporting the governments programs).
3. Cadres and the village authorities done the public awareness, early response, early detection and reporting bite cases to the local authorities independently.
4. Community showed positive response on Government programs and willingness to support the program.

RESULTS

Promising results from pilot villages model indicates high participation of the community (formed 82 cadres in Gianyar and Karangasem) which involved in public awareness, early reporting, early response and early detection on bitten cases activities. The positive response was showed also in government program particularly on the mass vaccination by registration data of the dog within community to achieved high coverage on vaccination (>70%).

Improvement the level of Knowledge

The public awareness which carried out in schools (6 elementary schools and 1 junior high school in Sibetan and 4 primary school in Melinggih Kelod) has covered 797 students elementary students and 1784 students in junior high school in Sibetan, while in the village of Melinggih Kelod has covered 362 students of elementary schools.

Based on the evaluation that conducted for two times (a week after and 3 months later) showed more than 52,% student for grade 4 in both villages has increased the knowledge, 50%, 48% for grade 5 and grade 6 respectively. The increasing knowledge has also showed for the junior high students with 81% for grade 7 and 64% and 75% for grade 8 and 9 respectively.

The improvement the level knowledge of the cadres and the community in both villages were not evaluated quantitatively. These were evaluated based on self-assessment of the cadres through focus groups discussion and the change of the behavior in both villages. The changes level of knowledge in the community is based on handling the bitten cases in the villages (that refers to procedure from standard health offices procedures), reporting of bitten cases to the cadres or local authorities and the level of enthusiasms on vaccination for their dogs. These activities were recorded by the cadres in the bitten cases book which will matched with rapid response report from the health offices and livestock offices in the district level.

High Coverage Vaccination

Based on door to door survey within the villages, the coverage vaccination revealed that both villages have high coverage (> 90%) vaccinations. This is positive result, If we compared with before the piloted program implemented (<80%).

Mass vaccination program was supported by dog registration by providing the data of the dogs in every banjar for the vaccinators. This made the vaccinator easier to identify and catch the dogs in every banjars since most of the dogs in Banjar were own dogs even though most of them are free roam.

Early Response, Early Detection, Reporting and Bite Case Management

In the end of community engagement program, the registration process in those villages registered approximately more than 80% of the village dog population. This data were used to facilitate vaccination, reduce unnecessary medical treatment on bite cases by promoting leashing and to check a dog's status after a bite.

As part of the early response, early detection, reporting and bite case management's efforts from the community. The cadres and also the village authorities' independently formalized a quick response system at the banjar level. In the case of a dog bite, information travelled from the community to the banjar leader to the head of the village and then to the livestock office and the working group committee to facilitate testing of the dog and proper medical care for the bite victim. This system reported 2 negative bite cases in one village and 14 negative dogs and 2 positive dogs in another village from September 2012 to March 2013. This system has showed to improve the early response and early detection on bitten cases and increasing the sensitivity of the surveillance by reporting to the local authorities (livestock offices).

During the process of this community awareness, the research team shared preliminary findings and helped priorities needs, plan activities, support implementation, and monitor the process. Community members identified a number of areas where they felt they could facilitate improvements, including the need to raise more awareness about rabies and facilitate

better care of dogs, improve surveillance of rabid dogs, maintain a dog registry, institute a *perarem* law (traditional law) and develop a quick response system. In both villages, community members self-organized into what they called a 'village rabies working group' involving religious leaders, government officials, policemen, medical doctors, veterinarians, teachers, village health workers and others. The working groups subsequently conducted a number of awareness raising activities at the monthly banjar meetings, at schools and other significant gatherings.

DISCUSSION

The rabies eradication targets from the Islands in 2015; bring some new challenges to the programs. Some of the concerns are maintaining the high level coverage of vaccination, dog population rebound, and increasing the sensitivity of the surveillance. The other concern is the level of awareness of the community since rabies cases have significantly reduced.

In terms of rabies vaccination, puppies and stray dogs are considered the most important targets in vaccination campaign and efforts to institute population management through sterilization, ending the practice of discarding unwanted females and the vaccination of puppies could all bead dressed through engaging such working groups. This need not be implemented in all areas of Bali, but could be used only where the rabies virus is known or suspected to be actively circulating.

Based on our finding we also found that the dog population will easily rebound due to plentiful availability of food waste and continued birth of puppies from fertile females. Population rebound will reduce levels of vaccination coverage through the addition of new susceptible animals in the population

The rabies eradication efforts from the island must focus heavily on strengthening surveillance capacity and rapid response as well as maintaining vaccination coverage. It is important to consider how the Balinese community could be more actively involved in optimising these efforts. There are both

strengths and challenges involved in instituting community participation in disease control programs that should be acknowledged (4, 6, 7). The piloted villages suggest that involving community members more actively in villages with recent rabid dog cases through community engagement could substantially improve surveillance activities with possible effects on improving dog management and vaccination coverage. With little direct financial input such participation creates a sense of community ownership that helps facilitate education, rapid response, the testing of suspected rabid dogs, dog registration and the use of traditional laws to motivate dog owners.

In the end a community based model for rabies control showed promising results indicates could be used effectively across other districts in Bali.

CONCLUSION

The effort of the Government on rapid response of bitten cases and dog bite management will be more effective if the community could be more actively involved in optimising these efforts.

Promising results from pilot villages' model indicates high participation of the community, effective early reporting, early response and early detection on bitten cases.

Dog registration activities could be used to facilitate vaccination, reduce bite cases by promoting leashing and to check a dog's status after a bite.

The community based- model could be increasing the sensitivity of the surveillance due to increasing on reporting to the local authorities.

The community based model-could be used as a model for rabies control effort.

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Influence of Vaccine Cold Chain Handling on the Protective Rate of Rabies Post Vaccination of Dogs in Western Sumatra

Heru Susetya[1*] Iis Irawanti[2] Setyawan Budiharta[1]

[1]Department of Veterinary Public Health, Faculty of Veterinary Medicine Gadjah Mada University, Indonesia
[2] Veterinary officer, Western Sumatra
*Corresponding author; Email: herususetya@ugm.ac.id

ABSTRACT Rabies is still highly endemic in Western Sumatra. The objectives of this study were to determine the state of the vaccine cold chain at service points of rabies vaccination in Western Sumatra and to investigate factors, including the vaccine cold chain, associated with the protective of antibody titer (≥ 0.5 IU/mL) of dogs in a case control study. Sample consisted of 90 cases (titer ≥ 0.5 IU per mL) and 90 controls (titer < 0.5 IU per mL). Potential risk factors were collected by means of interview and observation. The vaccine cold chain was assessed by observation and interview to the storage and vaccine carrier officers of the source of vaccine used. Associations between titer (protective or not protective) and potential risk factors were analyzed using chi-square and logistic regression. The result of cold chain assessment showed that many officers did not understand and did not follow properly the storage procedures. Some of the cold chain facilities and equipment were still inadequate. Factors significantly associated with the protective titer by chi-square test were the owner income, number of vaccination, vaccination programs (mass and door to door), intervals between the last vaccination and serum collection (<6, 6-12 and >12 months), vaccines cold chain, and locations. The model obtained by logistic regression analysis showed that more than one time vaccination, interval less than six months, the districts of Dharmasraya and Padang Pariaman increase the probability of protective titer while the mass vaccination program decrease it.

KEYWORDS: *case-control, rabies, vaccination, Western Sumatra*

INTRODUCTION

Rabies has spread in Western Sumatra since 1953, except for the Mentawai Islands. The eradication of rabies has been done by vaccination and population control. Vaccination as a major program conducted with the target at least 70% of the dog population in endemic areas in order to obtain sufficient herd immunity to rabies control (1). However in Western Sumatra herd immunity did not reach the expectation.

The objectives of this study were to determine the state of the vaccine cold chain at service points of rabies vaccination in Western Sumatra and to investigate factors, including the vaccine cold chain handling, associated with the protective of antibody titer of dogs.

MATERIALS AND METHODS

A case-control study was conducted using post-vaccination serological test data of dogs in Western Sumatra carried out by BPPV Regional II in years of 2010-2011. Antibody titer was measured by ELISA method (2). Samples consisted of 90 cases (≥ 0.5 IU per mL

titer) and 90 controls (< 0.5 IU per mL titer). Potential risk factors were collected by means of interview and observation.

The vaccine cold chain handling was assessed by observation and interview the storage and vaccine carrier officers of the source of vaccine used. Questions were including the aspects of personnel, facilities and equipments and vaccine cold chain procedures. The collected data were analyzed by univariate (descriptive), bivariate (using chi-square for the presence of associations and odds ratio to measure the strength of the association), and multivariate analysis (using logistic regression to determine the factors simultaneously influence the probability of a protective titer) using PASW Statistic 18 and Statistic for Windows Version 7 as program software. Multivariate analysis with logistic regression used by entering variables had p value less than 0.25. All statistical analysis used p level of 0.05.

RESULTS

Cold chain status of 21storage locations and 26carrier officers were evaluated and scored on. Generally, the vaccine cold chain handling and transport officers did not know the correct vaccine storage. They did not have knowledge because they have never attended the cold chain handling training and did not have cold chain guidebook even some of them have technical education (Figure 1).
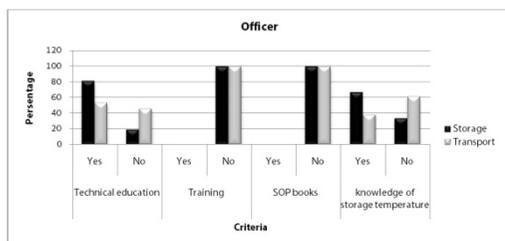
Figure 1: Description officer knowledge background on cold chain handling

There are also many officers who do not provide or prepare a cool pack to transport the vaccines. Like the storage officers, all the transport officers also did not have thermometer, so they never perform temperature measurements when carrying the vaccine.

DISCUSSION

Generally, the vaccine cold chain handling in the storage or transportation was not in improper procedures. Vaccines were arranged inappropriately inside the refrigerator. For the transportation, most of the vaccines were not well organized or placed in a separate container with frozen cold packs that all of the officer using for cooling. So, they can be damaged by cold shock and wet because of the ice melting. All officers used motorcycle to transport the vaccines so the carrier expose to the sunlight. Many of them also transport the vaccine more than four hours that was long enough.

The factors simultaneously influence the probability of a protective titer by logistic regressions was showed in Table 1.

The result showed that more than one time vaccination, less than six months interval since the last vaccination, Districts of Dharmasraya and Padang Pariaman increase the probability of protective titer while the mass vaccination program decrease it.

The effectiveness of vaccination in the districts of Padang Pariaman and Dharmasraya was very well. These two districts have a high positively influence on the probability of protective titers. The high influence of Dharmasraya and Pariaman districts may be due to several variables associated to the condition of protective titers in both of these areas.

The number of vaccination more than once has a positive influence on antibody titer. Antibodies will increase after the first vaccination, slow down and then increase again after the booster, reach a higher level than before, further down but still at a higher level than the first vaccination and leads to stable (3). The booster will increase the frequency to obtain a protective titer (5).Dogs sampled up to four or five months after vaccination are more likely to achieve protective antibody responses than those taken after that (4,6,7). The positively association of vaccination more than once and

Table 1: Factors simultaneously influence the probability of a protective titer by logistic regression.

Variable	Coefficient	S.E.	P	OR	C.I 95%		
Number of vaccinations	0,975	0,376	0.009	2,65	1,27	-	5,54
Mass vaccination programs	-1.116	0,355	0.002	0,33	0,16	-	0,66
Less than 6 month since the last vaccination	1,814	0,430	0.000	6,14	2,64	-	14,27
Dharmasraya	2,155	0,668	0,001	8,63	2,33	-	31,97
Padang Pariaman	3,233	0,926	0.000	25,36	4,13	-	155,92
Constant	-5,373	1,227	0.000				

The final model of the probability of a protective titer is showed the following equation.

$$P = \frac{e^{(-5,373 + 0,975\,(>1x)-\,1,116\,(\text{mass})\,+1,814\,(<6\;bln\;interval)\,+\,2,155\,(\text{Dharmasraya})\,+\,3,233\,(\text{Pdg.Pariaman}))}}{1+e^{(-5,373 + 0,975\,(>1x)-\,1,116\,(\text{mass})\,+1,814\,(<6\;bln\;interval)\,+\,2,155\,(\text{Dharmasraya})\,+\,3,233\,(\text{Pdg.Pariaman}))}}$$

interval of less than six months since the last vaccination with the protective titer indicates that, for rabies eradication program in Western Sumatra, revaccination after six months should be done.

The mass vaccination program was found to be a problem in achieving protective titer. The problems of implementation program might be related to some aspects like a bad vaccine cold chain, inaccurate vaccination procedures, and inadequate infrastructures. These things can become the focus of attention for increasing the success of vaccination.

CONCLUSION

Vaccine cold chain handling in Western Sumatra needs to be improve significantly in terms of personnel, facilities, equipment and procedures of storage. To achieve the protective post-vaccination rabies titers of dogs in Western Sumatra should be done revaccination after six months, an improvement of the implementation of vaccination programs and vaccine cold chain, and increase the client education to the dog owners about treatment that could support the formation of antibodies post-vaccination.

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Isolation and Identification of *Trichophyton mentagrophytes* from Dogs

Indarjulianto Soedarmanto[1,*] Yanuartono[1] Hary Purnamaningsih[1] Gerson Y.I. Sakan[2]

[1] Department of Internal Veterinary Medicine ,Faculty of Veterinary Medicine, University of Gadjah Mada, Jl. Fauna 2, Yogyakarta, 55281, Indonesia

[2] Program Studi Kesehatan Hewan, Politeknik Pertanian Negeri Kupang.

Corresponding author; E-mail: indarjulianto@yahoo.com, Tel.: + 62-274-560862, Fax. + 62-274-560861

ABSTRACT *Trichophyton mentagrophytes* is one cause of dermatophytosis in dogs that can be transmitted to humans, nevertheless information of this disease in dogs has not been reported in Yogyakarta, Indonesia. The aim of this study was to identify the *T. mentagrophytes* from the dogs dermatitis. Fifty-dog skin scrapings with clinical dermatitis lesions including alopecia, erythema, papules, pustules, scaly and crusty were used in this study. Identification of *T. mentagrophytes* are performed by macroscopic using modified sabouraud's dextrose agar (SDA) medium and microscopic using lacto phenol cotton blue (LPCB), followed by slide culture methods, and supported by urease test. The results of this study showed that 1 of 50 samples grew with white to cream colony on the upper side with granular to powdery colony. On the under side, it appeared a colony with cream – dark brown. The isolate was positive by urease test. Based on the results in this study, it can be concluded that 2% of dogs with dermatitis had *T. Mentagrophytes* which may transmit to humans.

KEYWORDS: Isolation, Identification, *Trichophyton mentagrophytes*, Dog

INTRODUCTION

Dermatophytosis is a superficial infection of the keratin tissues including nails, hair and stratum corneum of the skin in animals and humans caused by the dermatophytic fungi from the group of 3 genera, including *Microsporum, Trichophyton and Epidermophyton* (1, 2). The most common species of fungi isolated from hairs of dogs and cats are *Microsporum canis*, followed by *Microsporum gypseum* and *Trichophyton mentagrophytes* (1). *Trichophyton mentagrophytes* species commonly infects dogs, cats, mice and humans (3). Clinically infected symptoms caused by *T. mentagrophytes* are scaly and crusty and even seen a hardening of the skin (scaring) (3, 4). *Trichophyton mentagrophytes* is one cause of dermatophytosis in dogs that can be transmitted to humans. The population of pets and stray dogs in cities provides a possibility for contacts between animals and/or humans, which is a mode of transmission for some diseases. Fungal diseases in dogs caused by dermatophyte are zoonosis, contagious infections that affect dogs and cats, but also other animals, as well as humans. Nevertheless, information of this disease in dogs has not been reported in Yogyakarta, Indonesia. Our research was related to dogs with different changes on skin manifested by alopecia, erythema, papules, pustules, scaly and crusty. The aim of this study *was to isolate and to identify* the *T. mentagrophytes* from dogs dermatitis.

MATERIALS AND METHODS

The study was conducted on a number of 50 adult dogs and different sexes skin scrapings with clinically dermatitis lesions including alopecia, erythema, papules, pustules, scaly and crusty. Isolation and identification procedures refer to the manual of Beneke and Rogers (5), Al-Doory (6), Carter and Cole (7), Ates *et al*. (8), Ellis (9) and Seker

and Dogan (10). Samples of skin scrapings cultivated on Sabouraud's dextrose agar medium, incubated at a temperature of 25-30°C up to 21 days, and monitored every day. Identification of dermatophytic fungi growth was performed macroscopically and microscopically. Macroscopic identification carried out on a period of growth, colony morphology and color, shape, size and the rear of the colony. Urease test medium according to the guidelines Medical mycology manual (5, 6, 9) is used to distinguish the *T. mentagrophytes* from other strains (8, 10). Microscopic examination carried out on the observed positive fungal culture, using lactophenol cotton blue (LPCB). The result would be analysed descriptively from growth form of the fungal on the SDA and structure of fungal on the microscope, respectively.

RESULTS

Samples of skin scrapings obtained in this study originated from 50 dogs and then cultivated on SDA. After incubation of the SDA at a temperature of 25-28^0 C for 21 days, one sample grew as white-cream colonies with granular texture until powdery. At the bottom surface of them, the colonies looked beige to dark brown (Figure 1). Further urease test, the

Figure 1: Surface of a colony of *T. mentagrophytes*, 14 days after cultivation on SDA medium.

isolate hydrolyzed the urea. Macroscopically, the identification of colony by LPCB showed many grape-like microconidias with a single, clustered or arrayed structure along with spiral-shape hyphae. Some macroconidias could be found with thin walls and double

Figure 2: Microscopic structure of *T. mentagrophytes*; (a) macroconidia, (b) microconidia, (c) septae-hyphae with spiral shape.

septa with 4-6 cells (Figure 2). The rest samples grew differently with one sample grew earlier and had no ability to hydrolyze urea.

DISCUSSION

In this study there is one scraping sample with specific characters on macroscopic and microscopic examination and it is positive in urease test. These characters are identical with characters of *Trichophyton mentagrophytes*. According to Ates *et al*., (8); Issa and Zangana (11), the dermatophytic fungi can grow by incubation at a temperature of 25-30°C for up to three weeks. Macroscopically, topography of *T. mentagrophytes* is flat colonies with colonial texture that looks like a powder or granules with the surface color beige and white colonies. On the reverse side of the colony, the color is beige to dark brown (1, 5, 6, 9, 12).

The microscopic observations using dye LPCB, species *T. mentagrophytes* will show septae with hyphae. Microconidia is rounded in considerable amounts in the group conidiophores, single or arranged so as to resemble grape clusters along the spiral-shaped hyphae. Spiral hyphae is one of the key identification for these fungi. Spiral coil of hyphae are also frequently observed. Macroconidia in *T. mentagrophytes* which has a size of 20-50 mm x 4-7 mm was found in small amounts in some cultures, cigar-shaped with thin walls, smooth and has a double septum containing 3-6 cells (1, 6, 9, 12).

As the confirmatory test for the identification of *T. mentagrophytes* from other

strains, the urease test should be conducted to look for the ability of these fungi to hydrolyze urea. These fungi have the ability to hydrolyze urea (8, 11).

Trichophyton mentagrophytes is in a group of fungi that can zoophylically infect cats, dogs, rats and humans. In this study, there is only 2% of samples identified as *T. mentagrophytes* and this finding corresponds to data reported by Stojanov *et al* (13) that no fungi from *Trichopyton* from 81 of dogs samples were cultured.

However, Manelaos (3) reported that approximately 30% of dermatophytic infections in dogs are caused by the species *T. mentagrophytes*. This fungus is the most common cause dermatophytosis in dogs in India and the second most common in the United Kingdom (14). The disease is found in almost all types of pets. Dogs of all ages can be infected with dermatophytic fungi, but the incidence is more common in puppies. Besides age, other factors including poor nutrition status and management coupled by poor maintenance and lack of isolation by pet owners, will increase the incidence of this disease.

Disease mortality of dermatophytosis is low, however, the economic loss can occur due to damage in the skin and hair or weight loss because the animals become restless. Furthermore, it is a potential zoonotic disease, which can infect human (1). Prevalence of dermatophytosis always related to climate, temperature, humidity and precipitation, as well as the natural reservoir (15). The incidence Dermatophytosis generally increased in countries with hot climates or sub-tropical with a number of wild animals (2).

CONCLUSION

Based on the results in this study, it can be concluded that 2% of dogs with dermatitis had *T. mentagrophytes*, which may transmit to humans.

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Comparison of Bovine and Avian Purified Protein Derivatives on Bovine Tuberculosis in Chiang Mai Province (Thailand)

Tin Tin Lay[1,*] Anucha Sirimalaisuwan[2] Veerasak Punyapornwithaya[3]

[1] *LBVD, Ministry of Livestock Breeding and Fisheries, Myanmar and Master of Science (Veterinary Science), Faculty of Veterinary Medicine, Chiang Mai University, Thailand*
[2] *Department of Veterinary Bioscience and Veterinary Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Thailand*
[3] *Department of Food Animal Clinic, Faculty of Veterinary Medicine, Chiang Mai University, Thailand*
Corresponding author; Email: tintinlay7@gmail.com

ABSTRACT Bovine tuberculosis (bTB), caused by *Mycobacterium bovis* (*M. bovis*) is one of the economically important zoonotic diseases. Single intradermal tuberculin test or comparative interdermal tuberculin test can be widely used for test and slaughter control program. In Thailand, Department of Livestock Development (DLD) annually performs the tuberculin test with bovine purified protein derivative (bovine PPD). In this study, we compared the cell mediated antibody responses of bovine and avian (PPDs) in dairy cattle. The study was conducted in dairy farms of Mae Wang district in Chiang Mai Province. Seventy four cows were tested with comparative intradermal tuberculin test (CIDT). Bovine PPD was injected at caudal region and avian PPD was injected at cervical region. Skin thickness were measured before and 72 hours after injection. For each injection site, the thickness of skin before and after injection was compared using paired T-test. The sizes of the skin thickness for bovine and avian PPD before and after injections and differences of each injection site were compared using Welch's T-test. Result showed that measurements of skin thickness before and after injections were significantly difference ($P < 0.01$). There was significant difference comparing the difference in skin thickness for each injection site ($P < 0.01$). At bovine injection site, 90% of tested cows showed $\leq$ 2mm skin swelling difference and 6.8% illustrated 3mm, 2.7% produced 4mm respectively. Likewise in avian injection site, 82% showed $\leq$ 3mm, 15.2% produced 4-7mm and 2.7% illustrated more than 9mm respectively.

KEYWORDS: *Bovine tuberculosis, PPD (Purified Protein Derivative), Skin thickness*

INTRODUCTION

Bovine tuberculosis (bTB) is a chronic bacterial disease of cattle, human, other domestic animals and certain wildlife animals. It is caused by *Mycobacterium bovis* (*M. bovis*), a member of *Mycobacterium tuberculosis complex* (MTC) which is closely related to *Mycobacterium tuberculosis*(1). bTB is an OIE (World Organization for Animal Health) listed disease (2). It is an economically importance zoonotic disease and have challenge in cattle trading. In Michigan, US$ 7 million is used for bTB surveillance in cattle including indemnity payment, cleaning and disinfection of infected herds(3). In Asian region, only seven countries of 36 Asian countries apply test and slaughter

control program. bTB is partly controlled or not controlled in the remaining 29 countries in Asian countries. Ten countries didn't report bTB in Asian region (4). *M. bovis* in human continues to be reported in industrialized countries due to immigrants from the area where is endemic in bTB. In United State, approximately 1% of human tuberculosis is caused by *M. bovis*. The real incidence of *M. bovis* in human in developing countries underestimated due to inappropriate laboratory facilities (5). Global prevalence of human tuberculosis caused by *M. bovis* is 3.1% of all human TB cases(4). Even the risk to be negligible, it is still considered a public health risk. Other domesticated animals such as pig, horse, goat, sheep, dog, cat and certain wildlife population are susceptible to *M. bovis* (5, 6). Since bTB is the most complex and difficult multiple species endemic disease, the epidemiology is complicated and it is difficult to communicate the relationship between evidence, uncertainty and risk (7). Human to human aerosol transmission does occur in HIV endemic area and no practice in pasteurized milk consumption (5). Bovine tuberculosis infection in cattle is usually diagnosed in vivo on the basis of delayed hypersensitivity reactions. Tuberculin skin testis the standard method for detection of bTB. Single intradermal test with bovine PPD alone or comparative intradermal test with bovine and avian PPDs can be used in test and slaughter control program (6). The main objective of the study is to compare the cell mediated immune response of bovine and avian purified protein derivatives in comparative intradermal test in dairy cattle.

MATERIALS AND METHODS

Study area and period

The study was performed on May 2013 and the study population included the dairy cattle farms in Chiang Mai Province in the Northern part of Thailand. Chiang Mai Province is situated on the Mae Ping River basin and is 300 m above sea level, surrounded by the high mountain ranges of the Thai highlands. It covers an area of approximately 20,107 km². The study was carried out in Mae Wang district in Chiang Mai Province. This area is located between 18°47´43´´N and 98°59´55´´E. Cattle herds were selected due to geographic distribution and included after agreement by the farmers. Dairy cows in study area are more than 90% blood Holstein Frisian and open house type farming. In order to get more information, geographical coordinates registered at the cattle herd by hand held global positioning system (GPS) instrument.

Figure 1: Farm locations of the study area in Mae Wang District.

Comparative Intradermal Test

Seventy four dairy cows from three farms were tested with comparative intradermal tuberculin test. To avoid confounding effects, animals younger than 6 months, cows 1 month before and 1 month after parturition and clinically sick animals were excluded (8). Intradermal injection of 0.1ml containing 20000IU/ml bovine PPD (*M. bovis* strain AN5, Synbiotic, France) was administered at caudal fold. 0.1ml of avian PPD from *M. aviumsub sp. avium* strain D4ER (Prionics, the Netherlands, 25000 IU/ml) was injected at shaved site of cervical region. Skin swellings were measured before the injection was made and again 72 hours later. Pre-injection and post-injection of skin thicknesses were measured with the digital caliper. Measurements were carried out by the same researcher to avoid manual pressure.

Data analysis

We compared measurements of the skin thickness before and 72 hours after injections and also compared difference in skin swellings for each injection site. All data were entered into Microsoft EXCEL for data analysis. These

preliminary data keep for further analysis of this study. Recorded data were analysed using Statistical package R version 2.15.1.

RESULTS

In this study, we cannot interpret the positive reactor animal because of different injection sites. Comparing skin thickness before and 72 hours after injections for bovine and avian PPDs were significantly difference (P< 0.01). For each injection site, we also compare difference in skin swellings. There was significantly difference (P < 0.01). Seven cows showed ≥ 4mm skin thickness difference at each injection sites. At bovine injection site, 90% of tested cows showed ≤ 2mm skin swelling difference and 6.8% illustrated 3mm, 2.7% produced 4mm respectively. Likewise in avian injection site, 82% showed ≤ 3mm, 15.2% produced 4-7mm and 2.7% illustrated more than 9mm respectively. Two cows produced ≥ 4mm skin swelling difference on both PPDs. At both sites, more than 80% of tested cows showed between 2-4mm skin differences.
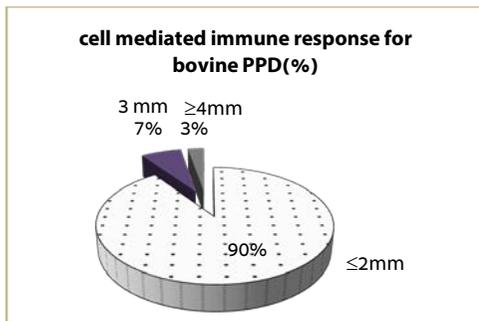
Figure 2: Percentage of skin thickness difference at bovine injection site.
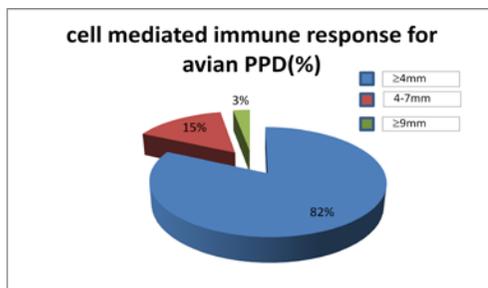
Figure 3: Percentage of skin thickness difference at avian injection site.

Percentages of cell mediated immune response on bovine PPD and avian PPD are illustrated in Figure (2) and (3).

DISCUSSION

In Thailand, DLD doesn't administer bovine PPD at cervical region because it is more convenient to do at caudal fold. As bovine and avian PPDs were injected at different regions, the interpretation cannot follow comparative intradermal test described in OIE guideline (6). The results show that no effect on bTB when comparing the cell mediated antibody response of bovine and avian PPDs. At bovine injection site, seven cows showed skin swelling differences between 3-4mm. Likewise in avian injection site, skin thickness differences before and after injections were ≥4 mm in seven cows. Two cows produced ≥4 mm skin swelling difference on both PPDs. This study described skin swelling differences were significant difference in statistically analysis even PPDs were not injected at the same region. A small number of cows showed greater size of skin differences at avian injection site.

CONCLUSION

This study proposed to compare cell mediated immune response of bovine and avian PPDS in CIDT test. There was no interpretation for positive reactor because of different injection sites. But in this study, skin swellings differences were statistically significant in pre and post PPD injection and at both sites. It can be concluded that *M. avium* infection has no effect on bovine tuberculosis.

ACKNOWLEDGEMENT

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Prevalence and Virulence-associated Gene Profiling of *Streptococcus suis* in Pigs Slaughtered for Consumption in Chiang Mai and Lamphun, Thailand

Wichanee Chanto[1,*] Duangporn Pichpol[2,3] Kanruethai Wongsawan[4] Reinhard Fries[5]

[1] *Joint Master Course in Veterinary Public Health (MVPH) of Freie Universität Berlin and Chiang Mai University, Thailand*

[2] *Division of Veterinary Public Health, Department of Veterinary Biosciences and Veterinary Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand*

[3] *Veterinary Public Health Centre for Asia Pacific (VPHCAP), Faculty of Veterinary Medicine, Chiang Mai University, Thailand*

[4] *Division of Veterinary Paraclinic, Department of Veterinary Biosciences and Veterinary Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand*

[5] *Institute of Meat Hygiene and Technology,* Faculty of Veterinary Medicine, *Freie Universität Berlin, Germany*

Corresponding author; Email: wichanee.c@gmail.com

ABSTRACT *Streptococcus suis* is now regarded as a zoonotic pathogen of concern in Thailand and elsewhere. The objectives of this study were to determine the prevalence of *S. suis* isolated from pigs slaughtered for consumption and genetic characteristics of this agent based on the presence of 3 virulence-associated genes; extracellular protein factor (*epf*), Muramidase-released protein (*mrp*), and Suilysin (*sly*). From a total of 220 tonsils from pigs slaughtered at 12 slaughterhouses in Chiang Mai and Lamphun during December 2012 to April 2013. *S. suis* was initially identified by microbiological techniques. Multiplex PCR targeting the gene encoding glutamate dehydrogenase (*gdh*) and serotype-specific genes of serotype 1 or 14, 2 or 1/2, 7, 9 16 was performed for confirmation and serotyping. The 3 virulence-associated genes were also investigated by Multiplex PCR. *S. suis* was isolated from 38.2% (84/220) of the tonsils collected. Serotype 16 (2.7%) was the most prevalent serotype followed by serotype 7 (2.3%), serotype 2 or 1/2 (1.4%) and serotype 9 (0.9%) respectively. Based on the presence of 3 virulence-associated genes, 7 various genotypes were found. Most of *S. suis* strains harboured the *epf- mrp- sly* - genotypes. This study demonstrated *S. suis* as a genetically diverse agent. Even though *S. suis* serotype 2 or 1/2 which is the most virulent strain causing human infections was found in only 1.4%, slaughter pigs are still considered an important key in the epidemiology of the infection due to its carrier capacity of various virulence-associated genes of *S. suis*. Awareness regarding *S. suis* infection needs to be extended to the public.

KEYWORDS: *Carrier, Pigs, Prevalence, Streptococcus suis*

INTRODUCTION

S. suis is one of the important pathogens widespread globally in pigs. In the last few years, the concern of *S. suis* infection in human has been raised significantly in many countries in particular Asian countries. Human can be infected with *S. suis* by consumption of contaminated raw pork or close contact with pigs or contaminated pig carcasses. In Thailand, most human cases have been found in the northern region by reason of eating raw pork or internal organs (1).

Usually, *S. suis* occurs as normal flora in pigs. However, *S. suis* can cause clinical

infection in any age of pigs but it is mainly found in young pigs after weaning. This agent colonizes primarily the upper respiratory tract of pigs especially in Tonsil and nasal cavities. Besides the respiratory tract, *S. suis* is also found in the genital and gastrointestinal tracts of pigs.

Healthy pigs are able to carry multiple serotypes of *S. suis*(2). It is considered to be a reservoir for *S. suis* infection in human.

Several factors have been inclined to explain the difference in virulence among *S. suis* strains (3). The association between difference in virulence and capsular polysaccharides (*cps*), muramidase-released protein (*mrp*), extracellular protein factor (*epf*) and Sulilys in (*sly*) had been reported (4).

Due to intensive pig farming in Chiang Mai and Lamphun, this area is considered a good representative area of the northern region of Thailand. The objectives of this study were to determine the prevalence of *S. suis* isolated from pigs slaughtered for consumption and the genetic characteristics of this agent based on the presence of 3 virulence-associated genes; *epf*, *mrp* and *sly*.

MATERIALS AND METHODS

Tonsil collection

From December 2012 to April 2013, tonsils were collected from 220 pigs slaughtered at 12 slaughterhouses including 7 slaughterhouses in Chiang Mai province and 5 slaughterhouses in Lamphun province. The total number of registered slaughterhouse in this area is 27 slaughterhouses.

After head removal, tonsils were removed aseptically from the head region of the carcass. Tonsils were placed in a sterile plastic bag individually and kept under $4^{\circ}C$ until microbiological analysis.

Bacterial isolation and identification

For surface decontamination, tonsils were submerged in boiling water for 1-2 seconds, cut into small pieces and placed in a sterile polyethylene bag. They were homogenized by stomacher. Afterward, a loopful of specimen was streaked on Blood Columbia agar (Oxoid®, UK) containing 5% (v/v) defibrinated sheep blood and Streptococcus selective supplement (Oxoid®, UK) and incubated at $37^{\circ}C$ in 5% CO_2 for 24 hr. Five suspectious colonies were sub-cultured on Tryptone Soya agar (Casein soya bean digest agar) (Oxoid®, UK) containing 5% (v/v) defibrinated sheep blood and incubated at $37^{\circ}C$ in 5% CO_2 for 24 hr.

The identification of suspicious colonies was initially based on the colony morphology, alpha-hemolysis on blood agar, gram staining, catalase test and biochemical characteristics including acidification of lactose, inulin, trehalose, mannitol and sorbitol, Esculin and arginine hydrolysis and Voges-Proskauer test. All suspicious colonies were further confirmed by Multiplex PCR.

Bacterial DNA preparation

S. suis isolates were cultured in Todd-Hewitt broth (Oxoid®, UK) at $37^{\circ}C$ in 5% CO_2 for 24 hr. DNA purification was performed by genomic DNA purification kit (ThermoScientific®, Lithuania) according to the manufacturer's instructions.

PCR-based identification for S. suis and serotyping

Multiplex PCR targeting the gene encoding the glutamate dehydrogenase (gdh) which is a specific sequence of *S. suis* was performed to identify the genus and species (5). In addition to gdh gene, the genes encoding the capsular polysaccharide (cps) were used to type *S. suis*. The serotype specific genes of serotype 1 or 14, 2 or 1/2, 7, 9 and 16 were amplified as described in the previous studies (5, 6).

The PCR reaction mixture (50 μl final volume) consisted of 25 μl of 2X Red dye master mix (Merck®, India) containing 1.5 mM $MgCl_2$, 0.1 μM of each primer and 3 μl of DNA template. The DNA of *S. suis* P1/7 serotype 2 strain was used as a positive control of gdh and cps2J genes. Nuclease-free water (ThermoScientific®, Lithuania) was used to replace the DNA template in the negative control. PCR amplification was carried out in a thermal cycler under the following condition; the initial denaturation for 2 min at $94^{\circ}C$, 35 cycles of denaturation for 1 min at $94^{\circ}C$, annealing for 1 min at $50^{\circ}C$ and extension for 1.30 min at $72^{\circ}C$ then the final extension for 10 min at $72^{\circ}C$. Amplified PCR products were

separated in a 2% Agarose gel, stained with ethidium bromide and visualized under UV light. A 100 bp DNA Ladder (ThermoScientific®, Lithuania) was used as a molecular size standard.

For confirmation of serotype specific genes, amplified PCR products of cps positive strains were sequenced and compared with the GenBank database using BLAST (blast.ncbi.nlm.nih.gov/Blast.cgi).

Virulence-associated gene profiling

The detection of virulence associated genes was performed by Multiplex PCR using the gene-specific primers including epf (7), mrp and sly (5). The DNA of *S. suis* P1/7 serotype 2 strain was used as the positive control of all virulence-associated genes.

mrp variant gene was detected separately by monoplex PCR as previously described (5).

Amplified PCR products were separated in a 1% Agarose gel, stained with Ethidium Bromide and visualized under UV light. 1 kb DNA Ladder (ThermoScientific®, Lithuania) was used as a molecular size standard.

RESULTS

From 189 suspicious isolates, 130 isolates were identified as *S. suis* by Multiplex PCR. *S. suis* was isolated from 84 of 220 tonsils (38.2%, 95% CI: 32.0-44.8) collected from pigs slaughtered for consumption during December 2012 to April 2013.

Serotype of 22 isolates were identified. Nevertheless, 108 isolates were non-typable strains.

Table 1: Percentage of *S. suis* isolated pigs slaughtered for consumption in Chiang Mai and Lamphun.

Serotype	Percentage of isolated pigs (No. of animals with serotype/total no. of animals)
2 or 1/2	1.4 (3/220)
7	2.3 (5/220)
9	0.9 (2/220)
16	2.7 (6/220)
Non-typable strain	30.9 (68/220)
Multiple serotype carrier	2.7 (6/220)

Serotype 16 (2.7%, 95% CI: 1.3-5.8) was the most prevalent serotype followed by serotype 7 (2.3%, 95% CI: 1.0-5.2), serotype 2 or 1/2 (1.4%, 95% CI: 0.4-3.9) and serotype 9 (0.9%, 95% CI: 0.3-3.3) respectively. Moreover,

pigs carrying multiple types of *S. suis* were found in 2.7 % (6/220) of pigs (Table 1).

For the presence of virulence-associated genes, the *epf*, *sly* and *mrp* genes were obtained from 3.8%, 10.0% and 20.8% of 130 *S. suis* isolates respectively. 7 various genotypes were found based on the presence of virulence-associated genes. Most of *S. suis* strains processed the *epf- mrp- sly* - genotypes (63.8%). 6 other genotypes were found as followed; the *epf- mrp+ sly-* genotype (16.2%), the *epf- mrp+ sly+* (8.5%), the *epf- mrp- sly+* genotype (6.2%), the *epf+ mrp- sly-* genotype (2.3%), the *epf- mrp*-varient+ *sly-* genotype (1.5%), the *epf+ mrp- sly-* genotype (1.5%) of the isolates. The distribution of virulence-associated gene profiling among different serotypes were investigated (Table 2). The difference of virulence-associated gene profiling was found among *S. suis* isolated from the same tonsil.

Table 2: Number of S. suis genotypes isolated from pigs slaughtered for consumption in Chiang Mai and Lamphun.

Genotype	No. of isolate with genotype/total no. of *S. suis* isolates
cps 2J	4/130
epf- mrp+ sly-	2/130
epf- mrp- sly-	2/130
cps 7H	6/130
epf- mrp+ sly+	5/130
epf- mrp- sly+	1/130
cps 9H	2/130
epf- mrp+ sly+	1/130
epf- mrp- sly-	1/130
cps 16G	10/130
epf- mrp- sly-	8/130
epf- mrp+ sly+	1/130
epf+ mrp+ sly+	1/130
Non-typable strains	108/130
epf- mrp- sly-	72/130
epf+ mrp- sly-	2/130
epf- mrp+ sly-	21/130
epf- mrp*+ sly-	2/130
epf- mrp+ sly+	4/130
epf- mrp- sly+	7/130
epf+ mrp+ sly+	2/130

DISCUSSION

In this study, *S. suis* serotype 16 was the most prevalent strain among 22 typable strains examined based on the detection of the *cps*16G genes. This result is different from a previous study (8) reported that *S. suis* serotype 2 was the most prevalent. However,

PCR-based identification for *S. suis* serotype 16 was revealed in 2011 (6).

S. suis serotype 16 was previously reported as a human pathogen (9).

Due to the limited identification of serotypes in this study, more than 80% of *S. suis* isolates was non-typable strains. This result suggested the identification for other serotypes should be done in further study.

Among 33 serotypes of *S. suis* that have been described, *S. suis* serotype 2 was reported as the most prevalent strain isolated from both diseased and healthy pigs worldwide. In Thailand, *S. suis* serotype 2 and serotype 14 had been frequently isolated from human cases than other serotypes (10). We found that the prevalence of *S. suis* serotype 2 or 1/2 was low (1.4%) during the study period, which was the case also in the study conducted in the eastern and western region of Thailand (11). *S. suis* serotype 14 was not found in pigs in this study.

However, PCR targeting *cps*1J and cps2J gene cannot differentiate *S. suis* serotype 1 or 14 and 2 or 1/2. Therefore, Slide agglutination using antisera is still an effective method for the identification of *S. suis* serotypes but it is laborious and expensive.

The most prevalent serotypes are geographically different. *S. suis* serotype 2 was the most common isolated from diseased pigs in France, Italy and Spain (12) which was similar to the investigation in China (13). However, serotype 15 and 9 were reported as the most prevalent later in Spain isolated from diseased and healthy pigs (14). Serotype 3 was the most common isolated in Korea (15) and the United states (16).

S. suis is a genetically diverse agent and the difference in virulence among *S. suis* strains was previously determined. Most of previous studies on virulence-associated genes have studied on *S. suis* serotype 2 strains. We carried out the study on all *S. suis* isolated from slaughter pigs. Most of *S. suis* isolates carried the *epf- mrp- sly-* genotype which was similar to previous studies in healthy pigs in Thailand and slaughter pigs in China (17, 18). The *epf- mrp+ sly-* genotype was mostly found in *S. suis*serotype 2 isolated from human cases

(17), which was also determined in this study. However, the presence of virulence-associated genes cannot definitely define the strain as virulent strain. Some non-virulent strains showed virulence factors, while some virulent strains without virulence factor were still virulent (3). Furthermore, *S. suis* has been considered as a pathogen with multi-factorial virulence factors (3) but the determination of virulent strain is not yet completely clear.

Slaughter pigs are still considered an important key in the epidemiology of the infection due to its carrier capacity for various virulence-associated genes of *S. suis*. Persons in daily contact with pigs especially those who work in the slaughterhouse need to be aware and prevent themselves by good personal sanitation and hygiene. Food safety education is also necessary for the effectiveness of preventing *S. suis* infection.

CONCLUSION

In this study, *S. suis* were isolated from pigs slaughtered for consumption in Chiang Mai and Lamphun and tested for genetic characteristics based on the presence of 3 virulence-associated genes. 7 various genotypes were found. Most of *S. suis* strains processed the *epf- mrp- sly -* genotypes. *S. suis* serotype 16 was the most prevalent among all typable strains which is different from the other studies in this area.

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First Findings from an Assessment of Domestic Slaughterhouse Operations and Postmortem Inspection under the DLD Slaughterhouse and Butcher Shop Improvement Project in Livestock Region 1 of Thailand

Suphanan Boonyakarn[1,*] Veerasak Punyapornwithaya[2,3] Maximilian Baumann[4]

[1] *Joint Master Course in Veterinary Public Health of Freie Universität Berlin and Chiang Mai University Thailand*
[2] *Department of Ruminant Clinic, Faculty of Veterinary Medicine, Chiang Mai University*
[3] *Veterinary Public Health Centre for Asia Pacific (VPHCAP), Faculty of Veterinary Medicine, Chiang Mai University*
[4] *International Animal Health, Faculty of Veterinary Medicine, Freie Universität Berlin, Germany*
Corresponding author; Email: suphanan63@yahoo.com

ABSTRACT In 2010, the Department of Livestock Development (DLD) set up the "Slaughterhouse and Butcher Shop Improvement Project" to help small and medium sized slaughterhouses and butcher shops which intended to upgrade their slaughter- and butcher facilities to become standardized in order to protect consumers better in terms of food safety and to provide enhanced support for meat inspectors to perform ante-mortem and post mortem inspection. The objectives of this cross-sectional study were to assess and monitor pig and poultry slaughter conditions, facilities for meat inspection and to record post-mortem inspection. A questionnaire and recording form were developed based on the DLD meat inspection form and on the new regulation "Ministry of Agriculture and Cooperative's Regulation on Determination of Criteria, Procedures and Conditions for Establishing the Slaughterhouse, Lairage and Animal Slaughter B.E. 2555 (2012)". The study was conducted during January – April 2013 in 5 selected slaughterhouses being 2 medium size pig slaughterhouses, 1 large size and 2 small size chicken slaughterhouses in Livestock Region 1 of Thailand. The post-mortem inspection facilities are not sufficient in terms of specific location, area space, suitability to inspect, availability of light and provision of hand washing by tap and/or hose. Post-mortem inspections were recorded for 30,195 chickens and 1,352 pigs slaughtered. For the pigs inspected, lung lesions and pleuritis were the most common lesion with 59.9%, followed by skin lesion with 14.6% and lesions at liver, spleen, heart and lymph nodes with 14%, 8.4%, 7.5% and 7.3%, respectively. The lesions found in chicken were 3.6% bruises/fractures, 1.3% arthritis, 1.2% skin lesions, 1.1% over-scalded and 0.3% were dead on arrival.

KEYWORDS: *Thai domestic slaughterhouse, Post-mortem meat inspection, Livestock Region 1*

INTRODUCTION

In 2010, the DLD set up the "Slaughterhouse and Butcher Shop Improvement Project" to help small and medium sized slaughterhouses and butcher shops which intended to upgrade their slaughter- and butcher facilities to become standardized and, thus, to build consumer confidence as well as to protect consumer in terms of food safety. The DLD project has four

areas of activity (i) ante-mortem and post-mortem inspection, (ii) certification of butcher shops, (iii) antimicrobial residues and microbiological quality analysis of meat from slaughterhouses and butcher shops, and (iv) public education and public relation (1). Since April 2011 DLD trained 190 meat inspectors and sent them to work at slaughterhouses in 63 provinces. These meat inspectors perform ante-mortem and post-mortem inspection, and are taking meat samples for laboratory testing for antimicrobial residues and bacterial contamination.

Thus, the study will observe pilot domestic slaughterhouses in this DLD project emphasizing on the conditions and facilities for meat inspectors and record the post-mortem meat inspection conditions and findings.

MATERIALS AND METHODS

Target slaughterhouses under DLD Slaughterhouse and Butcher Shop Improvement Project were selected from Livestock Regions 1, the middle part of Thailand; they must have regular slaughter and be accessible for observation. Based on these criteria, we selected 5 slaughterhouses, i.e. 2 medium size pig slaughterhouses, 1 large size and 2 small size chicken slaughterhouses.

Check lists and recording sheet to describe slaughterhouse facilities, operation and processing were developed based on the new regulation "Ministry of Agriculture and Cooperative's Regulation on Determination of Criteria, Procedures and Conditions for Establishing the Slaughterhouse, Lairage and Animal Slaughter B.E. 2555 (2012)" (2) and a post-mortem inspection recording sheet drafted.

Prior to field work proper, we visited the slaughterhouses, and field-tested the checklists, questionnaires, and the recording forms for post-mortem inspection. Due to non-suitable facilities and varying inspection practices, e.g. not consistently presenting all internal organs for palpation and/or incision, so post-mortem inspection data presented here were thus collected by visual inspection only.

Post-mortem inspections were recorded for at least 5 visits per slaughterhouse totaling 1,352 pigs at 2 pig slaughterhouses and 30,195 chickens slaughtered at 3 chicken slaughterhouses. Summary statistics were calculated in Microsoft Excel.

The field work was conducted between January – April 2013.

RESULTS

Table 1: A listing of study Slaughterhouses meeting building, process and inspection requirements in Livestock Region 1, Thailand.

	Slaughterhouses meeting criteria
General set-up at meat inspection point	
Working space satisfactory	2/5
Lighting sufficient	0/5
Appropriate working height	3/5
Facilities and equipment near the inspection point	
Reserve knife or knife sterilizer available	0/5
Water tap/hose for washing hands available	2/5
Condemnation container	
Available	5/5
Lockable	0/5
Speed of slaughter allowing proper inspection	3/5
Slaughter line not obstructed	2/5
Pig Inspection	
Viscera and head displayed and carcass paired with viscera and head for inspection	0/2
Chicken inspection	
Whole carcass inspection possible	0/3

From Table 1 it becomes apparent that for almost any criteria applied slaughterhouse compliance is deficient.

More than half of the pigs inspected presented inflammatory lesion in the respiratory tract but only every seventh pig liver was affected (Table 2).

In general PM findings in poultry were at a low level with bruises and fractures being most prevalent (Table 3).

Table 2: A first summary of pig post-mortem results (N=1,352) from the study slaughterhouses in Livestock Region 1, Thailand.

	Cases found	in %
PM findings in internal organs		
Lung lesions and pleuritis	810	59.9
Liver lesions	189	14
Spleen lesions	113	8.4
Heart lesion	101	7.5
PM findings in carcass		
Skin lesions	198	14.6
Lymph nodes abnormal	99	7.3
Wounds and abscesses	28	2.1
Trauma	22	1.6
Fractures	4	0.3

Table 3: A summary of chicken post-mortem results (N=30,195) from the study slaughterhouses in Livestock Region 1, Thailand.

PM findings	Cases found	in %
Bruises/fractures	1,097	3.6
Arthritis/joint lesions	407	1.3
Skin disease/lesions	363	1.2
Over-scalded	330	1.1
Emaciated/undersized	172	0.6
DOA (dead on arrival)	81	0.3
Hock burns	61	0.2
Breast blisters	33	0.1
Abnormal colour/septicaemia	23	0.08
Incomplete bleeding	9	0.03
Ascites	10	0.03

DISCUSSION

Our findings emphasize the necessity to improve certain facilities for meat inspection in slaughterhouses. The result shows that all 5 selected slaughterhouses have not adequate light for post-mortem meat inspection and not all poultry slaughterhouses inspect whole chicken carcasses. Inspecting carcass, viscera and head all the time is not possible at all pig slaughterhouses. Overall, three out of five slaughterhouses have limited space, no tap/hose water for washing hands and are not conveniently equipped to perform post-mortem inspection satisfactory.

Though the facilities for post-mortem meat inspection are covering only a small part of a slaughterhouse yet we found that mostly they are not suitable. In 2004, a similar study (3) showed that only around 10% of the licensed slaughterhouses complied with the Thai domestic slaughterhouse standard.

Even without additional financial investment, slaughterhouses could simply provide suitable areas of adequate height, light and water supply for hand washing thus facilitating inspection substantially. Hence, the government is expected to support items such as a flashlight for meat inspectors in their support for food safety control and prevention of disease from food animals to man.

The post-mortem inspections in pigs show that the majority of lesions are lung lesions and pleuritis with 59.9%, followed by skin lesions with 14.6%, liver lesions with 14%, spleen lesions with 8.4%, of heart lesions at 7.5% and lymph nodes lesions at 7.3%. In a previous study surveying one Thai pig slaughterhouse with 75,065 slaughtered pigs during January 2002 to September 2003 lung lesions were found to be 73% (4). In Lithuania, lesions detected during 2007–2009 were found in the respiratory tract with 78.98%, in the liver with 31.79%, the heart with 6.23% and the skin with 0.04% (5). A study in Austria during September 2007 to December 2010 in 264,039 slaughtered pigs found respiratory disorders such as pneumonia and pleuritis in 46.4% (6). The lung lesions in pig may have been caused by disease or infection, farm ventilation management or even have resulted from the slaughter process.

Post-mortem inspection in chicken showed lesions of 3.6% bruises and fractures, 1.3% arthritis/joint lesions, 1.2% skin disease/lesions and 1.1% over-scalded. The most frequent post-mortem findings in Sweden have been emaciation, discoloration, cellulitis (0.35%), ascites (0.27%), hepatitis (0.13%) and pericarditis in the flock (7). In a study in Algeria in 3 flocks of 3,000 broiler, the proportion of lesions found were congestion (30%), skin lesions (28%), cachexia (25%), ascites (8%), abnormal coloration (4%), arthritis (3%) and conformation (2%)(8). The high percentage of bruises and fractures in chicken results in economic losses from rejection or condemnation being mainly due to the handling of animals during the

slaughter process not in line with animal welfare.

This study used the visual meat inspection method and palpated and incised only in suspected cases. These post-mortem findings were recorded applying the DLD domestic meat inspection form modified to account for our additional criteria developed.

The new regulation "Ministry of Agriculture and Cooperative's Regulation on Determination of Criteria, Procedures and Conditions for Establishing the Slaughterhouse, Lairage and Animal Slaughter B.E. 2555 (2012)" came into force as from 4 July 2012 (2). Yet, this new regulation did not specify clearly enough the criteria for the post-mortem inspection facilities but mentioned that the slaughterhouse should delegate this to the officer.

CONCLUSION

These first results of the study point to the conclusion that the facilities for post-mortem inspection in the domestic slaughterhouses studied deserve substantial improvement. The post mortem inspection findings show that the majority of lesions in pigs are found in the lungs pointing to management failures in the farm of origin whereas the fractures and bruises in chicken were due to nonconformity with animal welfare during handling.

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Quantifying *Salmonella* Contamination in Pig Slaughterhouses in Hung Yen, Vietnam

Sinh Dang Xuan[1,2,*] Hung Nguyen-Viet[2] Tongkorn Meeyam[3,4] Reinhard Fries[5]

[1] *Joint Master Course in Veterinary Public Health (MVPH) of Freie Universität Berlin, Germany and Chiang Mai University, Thailand*

[2] *Center for Public Health and Ecosystem Research (CENPHER), Hanoi School of Public Health, 138 Giang Vo, Hanoi, Vietnam*

[3] *Department of Veterinary Biosciences and Veterinary Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Thailand*

[4] *Veterinary Public Health Centre for Asia Pacific (VPHCAP), Faculty of Veterinary Medicine, Chiang Mai University, Thailand*

[5] *Institute of Meat Hygiene and Technology, Freie Universität Berlin, Brümmer Str. 10, Berlin D-14195, Germany*

Corresponding author; Email: dxs@hsph.edu.vn, xuansinhck@gmail.com

ABSTRACT The risk of *Salmonella* contamination along the pork production is a worldwide concern. Not only the biological contamination, but also personal perception and individual behavior play a role for risk assessment along a chain. This study is to identify the prevalence and number of *Salmonella* in 3 pig slaughterhouses in relation to several risk factors. During January to April 2013, a total of 87 samples (carcasses, workers' hands, cutting board and belly skin material) were collected from 4 visits to each slaughterhouse. A 3-tube Most Probable Number (MPN) was applied to quantify the number of *Salmonella* from carcass, workers' hands, cutting board and belly skin samples (35 samples). The result showed that *Salmonella* prevalence was 36.9% (26.7-47.8%), mostly found on worker's hands (50%) and the lowest from cutting board (33.3%). The highest number of *Salmonella* on carcass and cutting boards was less than 0.075 MPN/cm^2 and 1.2 MPN/cm^2, respectively. Potential risk factors were tested however they were not statistical significant. This study underlines the necessity of good hygiene practices and management in slaughterhouses. In terms of food safety, further risk assessment of *Salmonella* in pork production chains is needed.

KEYWORDS: *Hygienic practice, pig slaughterhouse, risk factors, Salmonella, Vietnam*

INTRODUCTION

Salmonella is one of the main causes of foodborne illnesses in humans. Pork, after poultry meat, is a major source of human foodborne salmonellosis in the European Union (1). In Vietnam, from 2007 to 2011, 927 outbreaks of food poisoning occurred, with 30,734 cases and 230 deaths (2). Pork and pork products are most popular in Vietnam and distributed mostly by the traditional ways, i.e., small-scale slaughter, retail and pork producers where the risk of microbial contamination pork is relatively high.

Along the slaughter line, several steps can be critical with regard to *Salmonella* contamination: dehairing, removal of the intestines (3), washing, splitting the carcass. During these steps, the carcass can be contaminated with feces and bacteria can spread onto the carcass and to following

carcasses. Some studies on *Salmonella* prevalence in slaughterhouses in Vietnam showed that *Salmonella* contamination in water, on the floor, weighting bowls, cooking boards were between 16% and62%, on carcasses at slaughterhouse level ranging from 15% to 95%. At market, the *Salmonella* prevalence in pork was between 33 and 69% (4-5).

To understand the risk and magnitude of *Salmonella* contamination in a slaughterhouse, the objectives of this study were to identify the prevalence and number of *Salmonella* in pig slaughterhouses in Vietnam and to identify related risk factors.

MATERIALS AND METHODS

Sample collection

Pig slaughterhouses: Three pig slaughterhouses were selected in Hung Yen province including Van Giang, Van Lam and My Hao district. Each slaughterhouse was visited 4 times during January to April 2013. The capacity of these slaughterhouses was about 10-40 pigs per day with 4-6 permanent workers. Each visit was carried out in the early morning from 2 am till 5 am, sampling started after slaughter of 2-3 pigs and randomly for next samples.

Sample collection: In total, 87 samples were collected including 47 carcass swabs, 12 workers hands, 12 cutting boards and 16 samples by cutting tissue from the belly skin.

*Carcass swabs and belly skin samples:*400 cm^2 of split carcasses were swabbed at 4 different sites (hind limb-medial, abdomen-medial, mid-back and lower part of neck) (ISO 17604:2003). While skin samples were taken from belly, and put into sterilized plastic bag before transportation to laboratory.

Environmental samples: Worker's hand, cutting board swab samples were collected by using sterilized gauze during the processing. Two hands were swabbed on 2 sides and between the fingers. The cutting board was swabbed in an area of $25cm^2$.

Microbiological analysis

All samples were stored in a cool box and transported to the laboratory. 25 g of belly skin samples were taken and homogenized with 225 ml Buffered Peptone Water (BPW). Carcasses swab and environmental samples were added up to 100 ml BPW for homogenization. All homogenates were incubated at 37 ºC for 16-20 h.

Salmonella isolation: Salmonella was isolated from carcass and environmental samples based on ISO 6579:2002/Amd 1:2007 (6).

Salmonella Most Probable Number: Salmonella quantification was done by using the 3-tube MPN method (7). After 16-20h incubation at 37ºC, the *Salmonella* identification was performed according the previous mentioned procedure. The number of *Salmonella* positive tubes in each dilution was used to calculate the number of *Salmonella*(8).

Data collection

A checklist and a questionnaire were developed, tested and applied to obtain information at slaughterhouse level. The checklist was used to observe biosecurity, hygienic measures and technical practice in slaughterhouse. Questionnaires were applied to get about information of slaughtered pigs in each visit day.

Data analyses

Checklist and questionnaire were used for statistical analyses to test for related risk factors for *Salmonella* contamination. Odds ratio (OR), Chi square and Fisher' exact tests were used to determine significant factors leading to *Salmonella* positive result. R Studio (0.96.316) and MS Excel-2007 were used for data analyses.

RESULTS

General information of slaughterhouses

Result from questionnaires and recording sheets concentrate on variables linked to pig origin, transportation and lairage time. These results showed information from pre-slaughter stage of the 3 slaughterhouses (Table 1).

Information was obtained in relation to biosecurity, hygienic measures and technical practice.

Hygienic practice: Slaughter and processing in these slaughterhouses was done on the floor with limited separation areas for bleeding, scalding, or dehairing. All 3 slaughterhouses had an experienced worker assigned for evisceration. In 2 slaughterhouses, water stored in a tank was used to wash the floor, carcasses, hands and equipment. The other slaughterhouse used pipeline water directly to wash the floor, carcasses, and hands. All of the 3 slaughterhouses used biogas to treat the waste.

Table 1: Mean of variables recorded from pig transportation and lairage time.

Variables	Unit	n	Mean	SD	Min	max
No.of pig transport/time	head	12	23	7	17	40
Transport duration	hour	12	1.76	1.24	0.5	4.17
Transport distance	km	12	60.8	50.4	15	150
Time arrived at lairage	clock	12	13.9	4.17	10	22
Time spent in lairage	hour	12	13.1	4.17	5	17

For *biosecurity*, the 3 slaughterhouses carried out a separation of individual herds. Through 12 observations, abnormal signs, thin, or sick pigs were not seen in lairage.

Hygiene measures: One slaughterhouse had a separate entrance for pigs. People could access freely into the slaughter area. Workers frequently used boots, however they wore uniforms or apron rarely.

Salmonella prevalence

Overall, *Salmonella* was isolated from 36.9% (32/87) of all samples collected and from 34.9% (22/63) of carcass samples. *Salmonella* was mostly obtained from workers' hands, with 50% (6/12) and less frequently from belly skin samples, with 18.8% (3/16) (Table 2).

Quantification of Salmonella

The highest number of *Salmonella* on carcass and cutting boards was less than 0.075 MPN/cm^2 and 1.2 MPN/cm^2, respectively, and less than 0.3 MPN/g from belly skin. *Salmonella* enumeration from workers' hands ranged from 0 to 7.0 MPN/hand (Table 3).

Table 2: *Salmonella* prevalence from different sample types in 3 slaughterhouses.

Sample type	Prevalence_% (No. positve result/n)			
	SH A	SH B	SH C	Overall
Carcass surface	23.5 (4/17)	46.7 (7/15)	53.3 (8/15)	40.2 (19/47)
Belly skin	25.0 (1/4)	16.7 (1/6)	16.7 (1/6)	18.8 (3/16)
Workers' hand	25.0 (1/4)	100 (4/4)	25.0 (1/4)	50.0 (6/12)
Cutting board	25.0 (1/4)	50.0 (2/4)	25.0 (1/4)	33.3 (4/12)
Overall	24.1 (7/29)	48.3 (14/29)	37.9 (11/29)	36.9 (32/87)

SH A, B, C: Slaughterhouse A, B and C

Table 3: *Salmonella* number from different sample types in 3 slaughterhouses.

Sample types	Unit	No. of *Salmonella*	95% CI
Carcass surface	MPN/cm^2	<0.075	0.0-0.24
Belly skin	MPN/g	<0.3	0.0-0.95
Workers' hand	MPN/hand	0 - 7.0	1.0-50.0
Cutting board	MPN/cm^2	<1.2	0.0-3.80

Table 4: Combination *Salmonella* "qualitative" and observation as OR.

Factors	Level	OR (95% CI)	*p*-Value
Water tank	Yes	2.18 (0.67-7.07)	0.191
	No		
Slaughter quare	<50 m^2	1.67 (0.57-4.95)	0.350
	>50m^2		
Free entry slaughter area	Yes	2.18 (0.67-7.07)	0.191
	No		
Farm type (SH B)	Private	2 (0.29-13.74)	0.631
	Contracted		
Pig transport distance (SH C)	>30km	1.33 (0.2-9.08)	1.00
	≤30km		
Time arrived at lairage (SH C)	Morning	5.7 (0.53-61.41)	0.179
	Afternoon		
Disease in farm area (6 months) (SH A)	Yes	10.5 (1.03-107.2)	0.063
	No		

DISCUSSION

In this study, the overall prevalence of *Salmonella* on pig carcasses at slaughterhouse level (34.9%) was lower in comparison with other studies in Hanoi: 48.9%, by Thai (2007)

(10) and 95.7%, by Le Bas (2006) (4). Location and capacity of slaughterhouse might have been different. Findings in a study in Khon Kean by Sithigon (2011) showed slightly higher prevalence on pig carcasses (36.67%) but lower prevalence of *Salmonella* was found on workers' hands (10.71%) (11). Other review in Northern Thailand by Fries et al. (2006) described a lower prevalence of *Salmonella* on carcass swabs (33.2%) (12). The EFSA (2008) reported a lower *Salmonella* prevalence on pig carcasses in the EU (8.3%) (1), which might reflect the need for hygienic and management interventions at slaughterhouse observed in this study. There was no significant difference (p=0.403, Chi-squared test) among 3 slaughterhouses on *Salmonella* prevalence on carcass, which can be explained by the traditional way of slaughter on the floor with the need of hygienic improvement.

The low quantitative number of *Salmonella* on carcass surface, belly skin, and on cutting boards was not unexpected. The few available MPN-based studies also indicated that same low range. Among tested samples, workers' hands had contamination, ranging from 0-7 MPN/hand. Others samples gave the number of *Salmonella* were lower than limited detection of MPN table. Those were in accordance with findings of Boughton (2004) that the low numbers of *Salmonella* cells were typically found in food, feed and environment samples (13). Prendergast (2008) reported *Salmonella MPN* in environment pork cuts within a range of <0.03–0.36 MPN/g and in environmental swabs ranging from <0.03–1.1 MPN/cm^2 (14). Dehairing, scalding and washing minimized feces and dust. During slaughter, the amount of *Salmonella* load on workers' hands might come from touching pigs at many occasions (e.g., restraint, bleeding, scalding, evisceration, splitting) as well as coming into contact with the equipment. Even hand wash water might not be free of *Salmonella*.

The odds of *Salmonella* presence ranged from 1.3-10.5 (Table 4). The high OR related to the disease situation in farms for SH A might indicate an important point of attention in the stage of pre-slaughter. Fosse (2008) described

the need of characteristics of farms in order to distinguish among herds which one may be considered as high-risk herds (15).

Distance to the slaughter area, free entry of butchers or workers might impose a high risk of spread *Salmonella* from live pigs to the slaughterhouse environment (floor, hands, water, tools,…) which then may cross over to the carcass. Hurd (2002) showed that transport and lairage were important risk factors for *Salmonella* contamination (16). So, handling and hygiene practices are necessary to prevent cross contamination throughout the slaughtering process (17). Hald (1999) identified the floor as an important source of pathogens including *Salmonella* (18).

CONCLUSION

In this study, the prevalence of *Salmonella* in slaughterhouse was 36.9% and was mostly found on workers' hands. The number of *Salmonella* on carcass was lower than 0.075MPN/cm^2. These findings provide information on *Salmonella* contamination and related risk factors in slaughterhouses in Hung Yen. From those points, the effectiveness of hygienic practice and management in slaughterhouse should be properly applied to improve food safety and reduce the risk of foodborne diseases.

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Prevalence and Antimicrobial Resistance of *Salmonella* spp. in Slaughtered Pig in Pork Production in Chiang Mai and Lamphun, Thailand

Min Thit Lwin[1,*] Peter Paulsen[2] Duangporn Pichpol[3,4]

[1] *Joint Master Course in Veterinary Public Health (MVPH) of Freie Universität Berlin and Chiang Mai University, Thailand*

[2] *Institute for Meat Hygiene, Meat Technology and Food Science, Department of Farm Animals and Public Health in Veterinary Medicine, University of Veterinary Medicine Vienna*

[3] *Veterinary Public Health Centre for Asia Pacific (VPHCAP), Faculty of Veterinary Medicine, Chiang Mai University, Thailand*

[4]*Department of Veterinary Biosciences and Veterinary Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Thailand*

Corresponding author;Email:dr.minthitlwin@gmail.com

ABSTRACT A cross-sectional study was conducted to determine the proportion of antimicrobial resistance of *Salmonella* spp. of slaughtered pig in pork production in Chiang Mai and Lamphun provinces, Thailand during December 2012 to April 2013. A total of 111 caecal content samples were collected from 111 pig butchers for different flock of pigs, from 12 registered slaughter houses of Department of Livestock Development, Thailand. Caecal content samples were taken immediately during evisceration. *Salmonella* spp. was identified by the method slightly modified to ISO 6579:2002. Ten different antimicrobial agents belonging to 6 pharmacological groups including penicillin, tetracycline, cephalosporin, aminoglycoside, sulphonamide and quinolone were used for antimicrobial susceptibility test. Antimicrobial susceptibility test was carried out by the disk diffusion method of CLSI, 2012. *Salmonella* spp. was recovered from86.5% (96 in 111) .The most isolated *Salmonella* resistant to Ampicillin (84.4%, 94 in 111). No isolated salmonella resistant to norfloxacin (0%, 0 in 111). Moreover, this study found 92.7% (178 in 192) and 44 patterns of multiple antimicrobial resistances. Two isolated *Salmonella* were resistant to 8 antimicrobial agents and 6 isolates were susceptible to all 6 group antimicrobial agents. The study indicated that multiple antimicrobials resistant *Salmonella* spp. should be concern and publish to consumers, farmers and slaughterhouse's owners who get involved in pork production in Chiang Mai and Lamphun provinces.

KEYWORDS: *Antimicrobial susceptibility, Pig feces, Slaughter houses, Salmonella* spp.

INTRODUCTION

There are 16 million annual cases of typhoid fever, 1.3 billion cases of gastroenteritis and 3 million deaths worldwide due to *Salmonella* (1).

Salmonella Enterica is recognized as one of the most common causes of bacterial food borne illness worldwide. The majority of *Salmonella* infections are attributed to consumption of contaminated food of animal origin (7).

Pork is one of the most widely eaten meat in the world, accounting for about 38% of meat production worldwide, although consumption varies from place to place(15). Pork is also a staple meat in Thailand. The pork

consumption of Thailand in 2010 is 15.88 kg/capita/year (12) and pork production from of indigenous pigs was 936,425 metric tons(4). Chiang Mai and Lamphun provinces are the 5th highest swine density area and 1st highest local pig density area of Thailand(2).

The objective of the study is to determine the prevalence of antimicrobial resistance of *Salmonella* spp. in slaughtering pig in pork production of Chiang Mai and Lamphun Provinces, Thailand.

MATERIALS AND METHODS

Sampling

Random sampling has been done with 17 visits to 12 out of 23 pig slaughter houses registered by the Department of Livestock Development in Chiang Mai and Lamphun province during December 2012 and April 2013. Around 30- 40 g of different pig caecal content samples from different butchers were collected directly from caecum of the pig during slaughtering process and placed in sterile plastic bag. All samples were kept in ice box with ice packs. Microbiological analysis was done within 24 hours after sample collection. The general information as date, slaughtering batch and name of butcher were recorded.

Identification of Salmonella spp.

A slightly modified to ISO 6579:2002 *Salmonella* isolation procedure was applied for the all of the samples(9).

In brief, 25 g of feces were added to pre-warmed 225ml of Buffered Peptone Water (BPW) (Merck, Germany) and incubated at 37°C±1°C for 18h ± 2h. After incubation, 0.1ml was transferred to 10 ml pre-warmed Rappaport Vasiliadis (RV) (Merck, Germany) enrichment broth which was then incubated at 42°C for 24 h ± 3h, and 1 ml of broth was also transferred to 10 ml of Muller Kauffmann Tetrathionatenovobiocin broth (MKTTn) (Merck, Germany) for the secondary selective enrichment, with incubation at 37°C ± 1°C for 24 h ± 3 h.

From the secondary enrichment broth, a loop full of liquid from RV broth and MKTTn broth was streaked separately onto Xylose Lysine Tergitol-4 agar(XLT4 agar) (Merck,

Germany) and Brilliant green Phenol Red Lactose Sucrose agar(BPLS agar) (Merck, Germany) respectively. The plates were incubated at 37°C ± 1°C for 24 hr ± 3hr at inverted position and checked for the growth of typical *Salmonella* colonies in the next day.

Biochemical and serological confirmation

Five typical colonies per plate (XLT4 agar and BPLS agar) were picked and inoculated into Triple Sugar Iron agar (TSI) (Merck, Germany), Motility Indole Lysine medium(MIL)(Difco™, USA) Methyl Red VogesProskauer (MRVP) (Difco™, USA) broth and Urea agar (Himedia, India) and incubated at 37°C ± 1°C for 24hr ± 3hr.

The colonies which showed TSI (KA++), Indole (-), Motility (+), Lysine decarboxylation test (+), VP (-) and Urease test (-) were denoted as confirmed *Salmonella* spp. All positive isolates were further confirmation by slide agglutination with *Salmonella* O Polyvalent antisera (Serotest® Clinag, Thailand).

Antimicrobial susceptibility test

The antimicrobial susceptibility test was conducted following CLSI,2012(8).The confirmed *Salmonella* were picked up from the stock and streaked on the Nutrient agar (NA) (Oxoid®, UK), then incubated at 37°C for 18-24 hr. One or two well isolated colonies were picked up and transferred to the 3 ml of Tryptic Soya Broth (Oxoid®, UK) and incubated at 35°C for 4-6 hr until it reached the same turbidity as the 0.5 McFarland which equals to approximately $1-2 \times 10^8$ cfu/ml of *Salmonella* spp.

Sterile cotton swab was dipped in 0.5 McFarland of bacterial suspension and streaked thoroughly 3 planes on Mueller Hinton agar (MHA)(Difco™, USA).The antimicrobial disks (Oxoid®, UK) were tetracycline (30µg), doxycycline (30µg), Trimethoprim/sulphamethoxazole(25 µg), streptomycin (10µg), neomycin (30µg), gentamycin (10µg), ampicillin (10µg), cephalothin (30 µg), norfloxacin (10µg), orfloxacin(5µg). They were placed on prepared MHA and incubated at 37°C for 16-18 hr. Diameters of halos were measured as mm and the size of zone of inhibition was

interpreted by referring to zone diameter interpretive standard from CLSI, 2012(8) which equivalent minimal inhibitory concentration(MIC) break point for *Enterobacteriaceae*.

Data analysis

Data analysis was done by StataSE 9 software which was the license software of Veterinary Public Health Centre for Asia Pacific, Faculty of Veterinary Medicine, Chiang Mai University.

RESULTS

Out of 111 samples, *Salmonella* spp. was recovered from 86.5% (96) of the caecal contents of slaughter pigs which was slaughtered in 12 slaughter houses. The percentage of antimicrobial resistant *Salmonella* spp. against ampicillin, tetracycline, doxycycline, sulphamethoxazole/ trimethoprim, streptomycin, gentamycin, cephalothin, ofloxacin, neomycin, norfloxacin and were 84.4%, 79.2%, 75.5%, 40.1%, 34.9%, 13.5%, 10.4%, 7.8%, 5.7% and 0% respectively.
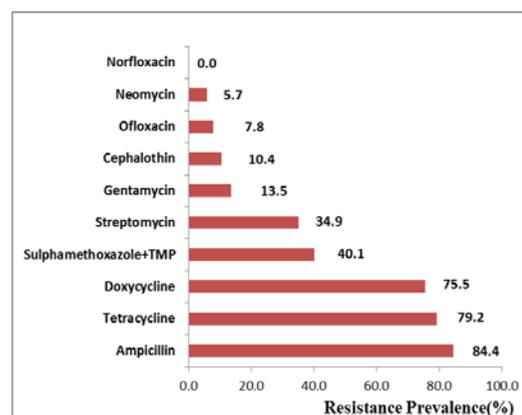
Figure 2: The percentage of *Salmonella* Resistant to 10 Antimicrobial Agents.

Out of 192 isolates 92.7% (178) were multi-drug resistant (MDR) with 44 different patterns. Major five MDR pattern was described in Table 1. Two isolates were resistant to 8 antimicrobial agents and 6 isolates were sensitive to all antimicrobial agents in the test.

Table 1: Major antimicrobial resistance patterns of *Salmonella* spp.

Pattern	Frequency	%	95% CI
AMP,TE,DO, SXT	36 in 178	20.2	14.7 - 26.5
AMP,TE,DO, S10	33 in 178	18.5	13.2 - 24.8
AMP,TE,DO	19 in 178	10.7	6.7 - 16.1
AMP,TE,DO,SXT,OFX	9 in 178	5.1	2.5 - 9.3
AMP,TE,DO,SXT,S10	7 in 178	3.9	1.7 - 7.9

AMP= Ampicillin, TE = Tetracycline, DO = Doxycycline, SXT =Trimethoprim/Sulphamethoxazole,S10 = Streptomycin, OFX = Ofloxacin

DISCUSSION

The prevalence of *Salmonella* spp. recovered from the caecal content of pigs during evisceration 86.5% in this study is clearly higher than the prevalence of 28% from the carcass swab samples from slaughter houses in 2002(6), 63% which sampling from feces of pre-slaughter pigs form one compartment in 2009(3)slightly higher than that of 83.4% from feces samples which was collected from only one slaughter house in2005(16)and that of 82% from carcass samples in 2013(11).

The prevalence of antimicrobial resistance of *Salmonella* spp. from feces sample of pigs against tetracycline 79.2% from feces samples is slightly lower than that of carcass swab samples 86%(6) and slightly higher than 77%(11)which was from destructed carcass samples. The resistant prevalence against to ampicillin 84.4% is clearly higher than 14% from the carcass swab samples and 51% from the destructed carcass samples (6, 11) but very close to 85% which was from the feces of fattening pigs (14)as compare to the previous studies. The resistance against to cephalothin 10.4% also significantly higher than the that of 5% in the previous study was from the feces of fattening pigs (14)and there is an increase of resistance prevalence of *Salmonella* spp. against on it. Even though 2nd generation of quinolone group norfloxacin was the most susceptible drug, *Salmonella* spp. already had resistance to ofloxacin in this study align with the report of quinolone resistant *Salmonella* spp. from other countries(5, 10).

The proportion of multidrug resistance (MDR) *Salmonella* spp. from feces samples 92.7% in this study was at high level as compared to the carcass swab samples from slaughter house 48% (13) and 50% (6) but very close to 91.74% from the feces and environmental samples of farm(14) . The high resistance to ampicillin and tetracycline caused in increase the multidrug resistance proportion in this study. Accordance with previous studies of the antimicrobial resistance patterns of *Salmonella* spp. from swine farms and abattoirs in Thailand, (AMP+TE+DO+SXT) and (AMP+TE+DO+S10) contributed as the major proportion of MDR in this study (11, 14).

CONCLUSION

In line with results of previous researches, the prevalence of *Salmonella* spp. was high in the pig feces in pork production in Chiang Mai and Lamphun with a significant incidence of multidrug resistance (MDR). The high prevalence of *Salmonella* spp. in pig feces was most likely come from the farms of origin, i.e, believed to be that the swine farms were threatened by multidrug resistant *Salmonella* and consequences to the public health. The high percentage of resistance against antimicrobial agents and the multidrug resistance problem was probably due to the misuse and abuse of Veterinary Medicine Products (VMPs) in Livestock production and might leads to obscurity in treatments for Salmonellosis.

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Microbiological Evaluation of Hygienic Practices of Pig Slaughterhouses in the National Capital Region, Philippines

Samuel Joseph Manglapus Castro[1,*] Tongkorn Meeyam[2,3] Reinhard Fries[4]

[1] *Joint Master Course in Veterinary Public Health (MVPH) of Freie Universität Berlin and Chiang Mai University, Thailand*

[2] *Department of Veterinary Biosciences and Public Health, Faculty of Veterinary Medicine, Chiang Mai University*

[3] *Veterinary Public Health Centre for Asia Pacific (VPHCAP), Faculty of Veterinary Medicine, Chiang Mai University*

[4] *Institute of Meat Hygiene and Technology, Faculty of Veterinary Medicine, Freie Universität Berlin*

Corresponding author; Email: samcastro_dvm@yahoo.com.ph

ABSTRACT   The continuing growth of the Philippine swine industry and intensification of swine production warrants the maintenance of effective and sustainable measures in slaughter facilities to ensure quality of products and prevention of disease spread. The purpose of this study was to evaluate and compare the hygienic status of two classifications of slaughterhouses, accredited and unaccredited, in the National Capital Region (NCR), Philippines through determination of hygiene indicator microorganisms. Environmental and carcass samples were collected and processed to obtain the Total Viable Count (TVC), enumeration of *Enterobacteriaceae* and detection of *E. coli* and coliforms for water samples, all in accordance to ISO standards. Both facilities were visited five times each from the period of January to April 2013 where a total of 190 samples (40 carcass swabs, 140 environmental swabs, 10 water samples) were collected and processed. The average TVC for all samples was 4.58 ± 1.25 log cfu/cm^2 whereas the overall value of *Enterobacteriaceae* was 2.08 ± 1.44 log cfu/cm^2 (n=180). The mean value obtained for TVC in pig carcasses (4.06 ± 0.95 log cfu/cm^2) was within the range set in guidelines being enforced in the Philippines. Likewise, the mean *Enterobacteriaceae* count on carcasses was 2.32 ± 1.48 log cfu/cm^2, within the acceptable range of those guidelines. Regardless of observable contrasts in terms of structure, equipment and facilities in both types of facilities, results from the study showed TVC and number of *Enterobacteriaceae* count in unaccredited and accredited facilities to be not statistically different in general. The largest number of coliforms (>2300 MPN/ml) and *E. coli* (2300 MPN/ml) were detected in a water sample from the unaccredited slaughterhouse while contamination in the accredited facility was found to be at lower levels. This study seeks to contribute to better understanding of the current hygienic conditions in such facilities, which would contribute to identification of gaps in the system and determine appropriate interventions for improvement.

KEYWORDS: *Hygiene, Slaughterhouses, Enterobacteriaceae, Philippines*

INTRODUCTION

Pork in the Philippines is a major commodity that has contributed greatly to the considerable growth of the country's livestock subsector of agriculture in the recent years. During the first quarter of 2012, the hog industry boosted the livestock subsector's growth with a recorded output increase of 3.42 percent (1). As such, swine production is considered the main livestock industry and is the largest contributor to meat output in the Philippines. The industry comprises about 58 percent of total meat output and is growing at 5.5 percent per year (3). In 2012, about 67 percent of the swine population was raised in backyard or small-hold farms and the rest were stocked in commercial production (1).

In the recent years, swine production in the Philippines has intensified in the urban and peri-urban areas in response to structural changes in the pig industry and largely due to a growing demand for pork products which is concentrated in the National Capital Region (NCR) or Metropolitan Manila, a major urban and commercial center (2). Arguably, NCR is the largest market for meat and meat products in the country.

Slaughterhouses that cater to urban areas such as those in the 16 cities and one municipality within NCR are of small to medium-scale and mostly for swine and some for bovines. Standards of these slaughter facilities, although in many cases not fully satisfactory, have improved over the years as the National Meat Inspection Service (NMIS) of the Department of Agriculture imposed a system of quality categorizing. Apart from those accredited by the NMIS, there is a large number of licensed slaughterhouses that amount to approximately four times that of accredited slaughterhouses (4). These slaughterhouses are licensed for commercial slaughtering by Local Government Unit (LGU) authorities (provincial, municipal/city) who oversee and who are responsible for sanitary control in these facilities.

Strict maintenance of good practices of slaughter hygiene in meat production is of vital importance for the prevention of microbial carcass contamination in the interest of ensuring both health protection and meat quality (7). There have been few and mostly unpublished studies done concerning slaughterhouse sanitation in the Philippines, let alone the area of concern in the study, the NCR. This study's objectives are to identify the number of hygiene indicator microorganisms in the selected slaughterhouses in the NCR and subsequently to compare the microbiological status between two classifications of slaughterhouses, accredited and unaccredited.

MATERIALS AND METHODS

The study assessed the microbiological hygienic conditions in two classifications of slaughterhouses in the NCR, Philippines: accredited and unaccredited. A survey on slaughterhouse operations and practices was conducted via administered questionnaire prior to sample collection to create a profile for each slaughter facility based on type, capacity and the slaughter process.

Sample Collection

In this microbiological investigation, environmental samples (swabs of scalding vat, de-hairing table, eviscerating/splitting table, floor, worker's hands, butcher's knife and splitting knife) and pig carcass samples were collected following Wet and Dry Double Swab Technique as described in ISO 18593: 2004(E). Each environmental swab covered an area of 25 cm^2 while for carcass samples, a total area of 100 cm^2 from 4 sites per carcass was swabbed using a non-destructive method as prescribed in ISO 17604:2003.

Both facilities were visited five times each from the period of January to April 2013 where total of 190 samples (40 carcass swabs, 140 environmental swabs and 10 water samples) were collected and processed.

Microbiological Analysis

Environmental and carcass samples were processed to obtain the Total Viable Count (TVC) and enumeration of *Enterobacteriaceae* in accordance to ISO 4833:2003(E), and ISO 21528-2:2004, respectively. Detection of *E. coli* and coliforms was done for water samples following a 10-tube Most Probable Number

(MPN) method following ISO 9308-2:1990. All samples where processed at the NMIS Central Meat Laboratory within an average of 5 hours after collection.

Data Analysis

Data management and organization was done using Microsoft Excel 2010. Statistical analysis of data was conducted using R Studio (version 0.96.316) for descriptive statistics (mean, standard deviation) and T-test for comparison of mean values.

RESULTS

Total Viable Count (TVC) and Enterobacteriaceae Count

The total mean value for TVC for all swabs collected in both facilities was 4.58±1.25 log cfu/cm^2 (n=180). For environmental samples on the whole, the total mean was 4.73±1.29 log cfu/cm^2 (n=140) while a value of 4.06±0.95 log cfu/cm^2 (n=40) was recorded for all carcass samples (Table 1).

Table 3: Total Viable Counts and *Enterobacteriaceae* counts in environmental and carcass swabs.

	Samples	n	Mean±SD (log cfu/cm^2)
Total Viable Count	Environmental samples	140	4.73±1.29
	Carcass samples	40	4.06±0.95
	All Samples	180	4.58±1.25
Enterobacteriaceae Count	Environmental samples	140	2.01±1.42
	Carcass Samples	40	2.32±1.48
	All Samples	180	2.08±1.44

The total mean *Enterobacteriaceae* count for the all swabs obtained was 2.08±1.44 log cfu/cm^2 (n=180). Environmental samples and carcass samples had means of 2.01±1.42 log cfu/cm^2 (n=140) and 2.32±1.48 log cfu/cm^2 (n=40), respectively (Table 2).

Comparing the two classifications, the mean TVC of environmental samples in the unaccredited slaughterhouse was 4.51±1.06 log cfu/cm^2 (n=70) and that from the accredited slaughterhouse was 4.95±0.63 log cfu/cm^2 (n=70). This difference was statistically significant. In carcasses, TVC means were 3.99±0.91 log cfu/cm^2 (n=20) and

4.13±1.01 log cfu/cm^2 (n=20) in the former and latter facilities, respectively (Table 2).

The mean value for *Enterobacteriaceae* was of 2.03±0.78 log cfu/cm^2 (n=70) for all environmental samples from the unaccredited abattoir, slightly higher than the accredited facility at 1.99±0.64 log cfu/cm^2 (n=70). For carcasses, counts were 2.12±1.02 log cfu/cm^2 (n=20) and 2.53±1.84 log cfu/cm^2 (n=20) in the former and latter facilities.

A comparison of means of the various environmental sites along the slaughter lines of both the unaccredited and accredited facilities is demonstrated in Table 3 for TVC and Table 4 for *Enterobacteriaceae* count.

Water Samples: Most Probable Number (MPN) of E. coli and coliforms

The number of coliforms and *E. coli* detected in water samples from the unaccredited abattoir ranged from 4.51 to >2300 MPN/ml and 2.71 to 2300 MPN/ml respectively (Figure 1).

For the accredited facility, the range of coliforms detected was <0.90 to 16.67 MPN/ml while *E. coli* levels were from <0.90 to 11.61 MPN/ml (Figure 2).

Figures 1 and 2 not scaled uniformly to demonstrate and emphasize the range of values obtained for each facility.

DISCUSSION

Results indicate the average total bacterial load for all samples at 4.58±1.25 log cfu/cm^2 whereas the overall value of *Enterobacteriaceae* was 2.08±1.44 log cfu/cm^2 (n=180), the latter being slightly lower than in a similar study conducted in slaughterhouses in Hanoi, where an average of 2.7 log cfu/cm^2 was found (5).

The analysis of data gathered from the study revealed total bacterial load and levels of hygiene indicator microorganisms to be within acceptable levels. The mean value obtained for TVC in pig carcasses (see Table 1) was within the value set in Philippine guidelines (4.0 – 5.0 log cfu/cm^2) (6). Similarly, the mean count for *Enterobacteriaceae* in carcasses (see Table 1) was within the acceptable range of 2.0 – 3.0 log cfu/cm^2 (6).

Table 4: Comparison of mean values for TVC and *Enterobacteriaceae* count in environmental sites and carcasses from both unaccredited and accredited slaughterhouses.

		n	Unaccredited (log cfu/cm²)	n	Accredited (log cfu/cm²)	*p-value*
TVC	Environmental samples	70	4.51±1.06	70	4.95±0.63	*0.0434**
	Carcass samples	20	3.99±0.91	20	4.13±1.01	*0.6497*
EC	Environmental samples	70	2.03±0.78	70	1.99±0.64	*0.851*
	Carcass samples	20	2.12±1.02	20	2.53±1.84	*0.3806*

statistically significant with p-value <0.05

Table 5: Comparison of mean values for TVC in environmental sites sampled in unaccredited and accredited slaughterhouses.

Sampling Site	n	Unaccredited (log cfu/cm²)	n	Accredited (log cfu/cm²)	p-value
Scalding Vat	20	2.57±0.87	20	4.22±0.70	*0.0002**
Dehairing Table	20	4.41±1.84	20	5.46±0.38	*0.1061*
Eviscerating Table	20	5.64±0.92	20	5.53±0.44	*0.7520*
Floor	20	5.76±0.71	20	5.20±0.72	*0.0956*
Butcher's Knife	20	4.64±1.50	20	5.56±0.40	*0.0900*
Splitting Knife	20	4.22±1.40	20	4.21±0.85	*0.9919*
Workers Hands	20	4.32±1.50	20	4.46±0.88	*0.7974*

statistically significant with p-value <0.05

Table 6: Comparison of mean values for *Enterobacteriaceae* counts in environmental sites sampled in unaccredited and accredited slaughterhouses.

Sampling Sites	n	Unaccredited (log cfu/cm²)	n	Accredited (log cfu/cm²)	p-value
Scalding Vat	20	1.03±1.38	20	1.22±1.40	*0.7603*
Dehairing Table	20	1.60±1.46	20	1.38±1.39	*0.7307*
Eviscerating Table	20	1.20±1.09	20	1.49±1.32	*0.5882*
Floor	20	2.47±1.43	20	2.27±1.36	*0.7507*
Butcher's Knife	20	3.11±1.52	20	2.32±0.90	*0.1810*
Splitting Knife	20	2.68±0.86	20	2.21±1.86	*0.4851*
Workers Hands	20	2.15±0.50	20	3.01±1.41	*0.0942*

The study compared mean values of TVC and *Enterobacteriaceae* count in environmental sites and in pig carcasses between unaccredited and accredited slaughterhouses. Results show that the difference between the mean TVC of environmental samples in the unaccredited
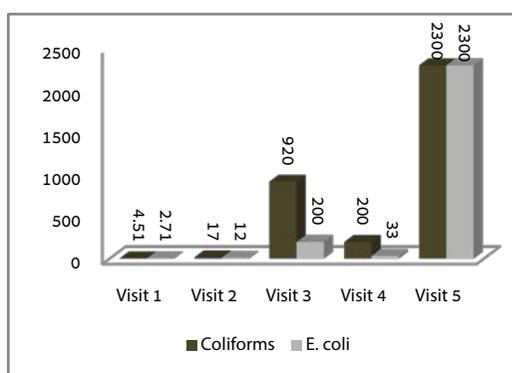
Figure 3: Most Probable Number (MPN) of coliforms and *E. coli* in water samples from an unaccredited slaughterhouse in NCR.
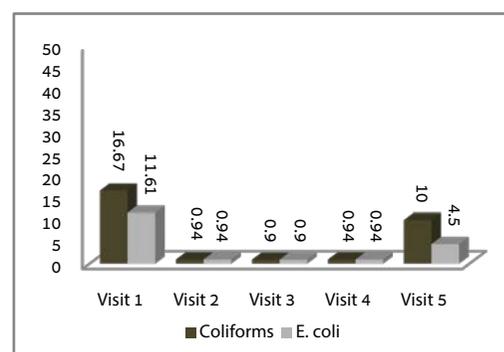
Figure 4: Most Probable Number (MPN) of coliforms and *E. coli* in water samples from an accredited slaughterhouse in NCR.

slaughterhouse and that of the accredited slaughterhouse was statistically significant (see Table 2). This is also reflected in comparing TVC and *Enterobacteriaceae* counts of each individual environmental sample, where there was a statistically significant difference in the TVC means of scalding vats from the unaccredited and accredited facility (see Table 3). This can, however, be attributed

to the irregular slaughter schedule of the former where most of the time, the scalding vat had already been pre-heated prior to swabbing. Meanwhile, the difference between means of samples for TVC in carcasses or *Enterobacteriaceae* in both carcasses and environmental samples were not statistically significant (see Tables 2 and 3). Therefore, despite observable differences in terms of structure, equipment and facilities, results from the study showed TVC as well as number of *Enterobacteriaceae* both types of slaughterhouses to be not statistically different in general.

Problems may also spur from unhygienic practices tolerated by the management. This is evident in the high level of contamination of coliforms and *E. coli* in water from the unaccredited slaughterhouse and the occurrence of contamination, albeit at lower levels, in the accredited facility. Among water samples obtained, the largest number of both coliforms and *E. coli* were detected in samples from the unaccredited slaughterhouse (see Figure 1). The cause of such values is probably the practice of gathering water in a fixed tub in the slaughter line which is the source of water for any and all activities in the facility from washing of hands, rinsing of knives, up to the final wash of the carcass. The water is replenished at the end of each operation and the tub is drained following no regular schedule. On the other hand, the least number was detected in accredited facility at with <0.90 MPN/ml in both coliform and *E. coli* counts. However, coliforms and *E. coli* were detected on two occasions in the said facility (see Figure 2) at levels higher than that prescribed in the country standards. This emphasizes a need from improvement of water quality and hygiene management in both facilities.

CONCLUSION

This study has provided insight that may contribute to better understanding of the current hygienic conditions in unaccredited and accredited slaughterhouses in the region. In the process, this study has as well identified gaps which will help to determine appropriate interventions that may be taken for improvement.

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A Study of the Occurrence of *Alaria alata* Mesocercariae in Pig Carcasses in Nine Provinces Bordering Mekong River, South of Vietnam

Duyen Thuy Thanh Phan[1,*] Lertrak Srikitjakarn[2,3] Saruda Tiwananthagorn[2] Phuong Thi Thuy Thai[4] Peter Paulsen[5]

[1] *Joint Master course in Veterinary Public Health (MVPH) of Freie Universitat Berlin and Chiang Mai University, Thailand*
[2] *Chiang Mai University, Thailand*
[3] *Veterinary Public Health Centre for Asia Pacific (VPHCAP), Faculty of Veterinary Medicine, Chiang Mai University*
[4] *Regional Animal Health Office No. 6, Vietnam*
[5] *University of Veterinary Medicine Vienna, Austria*
Corresponding author; Email: duyenphan1982@gmail.com

ABSTRACT *Alaria* sp. is considered a potential zoonotic trematode, because an intermediate stage of this parasite has been implicated in human disease, although sporadically. As regards *A. alata*, final hosts of the parasite are (wild) carnivores. The intermediate hosts are wetland- or water-associated, e.g. snails and tadpoles/frogs, but a number of vertebrates can act as paratenic hosts of the mesocercarial stage of the parasite. From a historical perspective and considering food safety aspects, pigs have been identified as carriers of *Alaria* mesocercariae. These parasitic stages can be recovered from adipose tissue, salivary glands and skeletal muscles. *Alaria* spp. has been reported in America and Europe, but information from Asian countries is scarce. Based on the biology of *A. alata*, this study is taken as a pilot survey to investigate the occurrence of *A. alata* mesocercariae in fresh pork in Mekong Delta, South of Vietnam where the river density is high and the pig traditional farming has low bio-security. The samples were collected from pigs originating from the backyard farms at slaughterhouses in provinces bordering the branches of Mekong River from December 2012 to May 2013. The cheek and peritoneal tissue samples were collected from 621 pig carcasses immediately after slaughter process. The samples were tested by the "Alaria Migration Technique (AMT)" developed by Riehn *et al*. (2010). None of the samples tested positive, indicating that if *Alaria alata* is present in the population studied, the prevalence of infected pigs is less than 5% during the sampling period.
KEYWORDS: *Alaria mesocercariae, pig, Vietnam, Alaria-Migration-Technique*

INTRODUCTION

The public is increasingly concerned about food safety since every day "new" diseases seem to emerge. The concerns are not only about hazards to human health, but also about economic losses and food security implications. Supporting to the public concerns, modern machines, new techniques and methods are developed. Thus, some new agents are found by accident or some agents ignored in the past to become hot topics in science communities and the worldwide public today.

In aspects of food safety and zoonosis, *Alaria* sp., in particular *A. americana*, can be the reason for severe damages in humans. Several reports of human larval alariosis indicate that infected game animals are a

potential source of infection for humans, but also animals(1-6, 8, 18, 20). Another *Alaria* species, *A. alata*, can exist in the intestines of carnivores worldwide. The cycle life of *Alaria alata* is not so clear until now. It involves two intermediate stages in intermediate hosts. The first stage, *Alaria alata* sporocysts can be found in snails (Family Planorbidae)(12). The second stage, mesocercariae, is found not only in the regular intermediate hosts, as amphibia/tadpoles(17), but also in a range of vertebrates, amongst them also pigs and wild boars ("paratenic hosts"). Risk factors for pigs being infected with mesocercariae are: (a) density of waterways, ponds etc. (i.e. presence of a habitat suitable for intermediate hosts); (b) low bio-security in pig farming, (c) (wild) carnivores carrying the adult parasite. It is not proven that *Alaria alata* mesocercariae can cause human illness; however, the presence of this parasite in edible porcine tissues is undesired anyway.

Data on the prevalence of the adult form in the definitive hosts are yet lacking and both environmental factors as well as the animal husbandry system in Vietnam may create a niche for this parasite such as the density of watercourses being very high in whole country Vietnam, special in the Mekong Delta in the southern part of Vietnam.

The animal keeping is not so well organized. In the southern part of Vietnam, small-scale swine husbandry is very popular. Pig pens are built near rivers, ponds or canals, not always with a solid bottom. The animals are not kept separately from human and direct contact between different species such as swine, dogs, cats and human, is possible. Quality of the water supply for pigs is not always controlled.

There is no data about the presence of *Alaria* spp. in Vietnam nowadays. So, a study is undertaken to obtain data on the occurrence of *Alaria* spp. (*A. alata*) mesocercariae in swine population of nine provinces bordering the Mekong Delta, South of Vietnam. Moreover, the information is expected to link the data on occurrence of mesocercariae to potential risk factors in traditional swine farming in the area.

MATERIALS AND METHODS

Place of study

Provinces bordering Mekong Delta, South of Vietnam areLong An, Tien Giang, Ben Tre, Dong Thap, Can Tho-Vinh Long, Tra Vinh, An Giang, Soc Trang, Kien Giang (Fig. 1). Pigs will originate from farms where they have close contact with ponds/rivers/watersheds (i.e. biotopes where the intermediate hosts could occur). It was planned that samples are collected at the largest abattoirs in nine provinces bordering Mekong River, Southern of Vietnam.
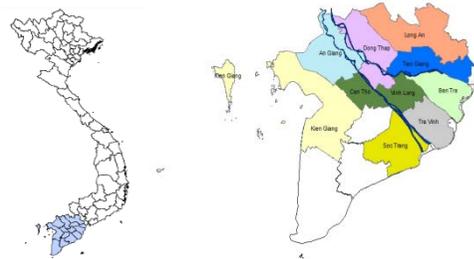
Figure 1: *Sampling Provinces of Mekong Delta in Southern, Vietnam.*

Sample size and sampling method

The study was designed to detect *Alaria alata* mesocercariae in pig carcasses in provinces bordering Mekong River. The sample size was calculated for each province with estimated prevalence 5% (p), 95% C.I., accepted error 5% following the formula:

$$n = (1 - (1 - p)1/d) \times (N - d/2) + 1 \quad (22)$$

The sample size was adjusted with expected sensitivity and specificity of test are 85% and 100%, respectively. Thus, the sample size per province was adjusted to 69. So the total number of tested carcasses was 621.

In general, the farm size is small scale in that the pig keeping was less than 100 pigs/farm in Mekong Delta (85% pig population)(7).The samples were collected from pigs come from the small scale and the number of the pigs came up from 90% to 100% at slaughterhouses(13, 14). Slaughtered pigs population of each province is described in table 1 below.

Table 1: Slaughtered pig population distribution at province level, 2012.

No.	Province	No. of Slaughtered pig(head)
1	Tien Giang	645,569
2	Ben Tre	396,952
3	Can Tho - Vinh Long	569,442
4	Đong Thap	267,495
5	An Giang	160,516
6	Kien Giang	294,729
7	Soc Trang	264,456
8	Tra Vinh	406,484
9	Long An	283,455

Within each province, the largest slaughterhouses were visited for sampling. If there was no central slaughterhouse, 2 largest slaughterhouses were selected. The total of slaughterhouses (SH) included in this study was 15 and the slaughterhouse size was designed to slaughter from 50 to 500 pigs/night. In each slaughterhouse, on the days visited, samples were taken from

Table 2: Calculation of Sample size by province.

No.	Province	Sampled SH	Sample
1	Tien Giang	1	69
2	Ben Tre	2	69
3	Can Tho - Vinh Long	3	69
4	Đong Thap	1	69
5	An Giang	2	69
6	Kien Giang	2	69
7	Soc Trang	2	69
8	Tra Vinh	1	69
9	Long An	1	69
	Total	15	621

the carcasses coming from the districts belong to that province. This meant that in total nine provinces were sampled (table 2), and within provinces, different origins of pigs (communes, districts) were considered. Total of 621 was randomly selected from pigs raised in small scale farms.

Sample isolation method

Based on the visualization by stereomicroscope demonstrated that parasites were very motile and the parasite (a) shows a high affinity to liquids and (b) moves actively out of the tissues, a new migration technique (AMT) was developed basing on a larvae migration technique by Riehn *et al*. (2010)(15).

The peritoneal and cheek tissues were mixed, chopped to 5mm size cubes and 30g of these tissues were tested by Alaria Migration Technique within 7 days after sampling.

RESULTS

Total 621 samples were tested by *Alaria mesocercariae* Migration Technique, by Riehn *et al*.(2010)(15). There was no *Alaria* spp. mesocercariae found in the samples.

DISCUSSION

During the study period, some visits were undertaken to the areas where the intermediate hosts of first stage of *alaria* should be expected. In the rice fields, some chemicals (such as niclosamide, metaldehyde products) were used to protect the crops that can kill snails also. This may interrupt the life cycle of the parasite. Besides, the moving ducks were raised in the rice field. It can reduce the snail population.

The traditional farming system of pigs in Mekong Delta is of low bio-security and the natural environment can be a niche for the parasite. Having consulted, local para- vets, the farmers applied deworming by praziquantel products. It can hide the real situation if the parasite mesocercariae is present in the population.

Finally, the sensitivity of the method of detection or difficulties in identification of presumptive mesocercariae could have contributed to the lack of "positive" results. However, from Riehn et al. (2010)(15), it can be concluded that such difficulties are unlikely unless mesocercariae would have been devitalized in the sample prior to analysis (e.g. by deep-freezing).

CONCLUSION

Based on the sample size used would, if *Alaria* spp. is present at all, be less than 5% in the population studied.

ACKNOWLEDGEMENT

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Prevalence and Antimicrobial Resistance of *Vibrio* spp. in Retail Shrimps in Hanoi, Vietnam

Tra Vu Thi Thu[1,*] Duangporn Pichpol[2,3] Ngan Pham Hong[4] Stephan Huehn[5] Thomas Alter[5]

[1] Joint Master Course in Veterinary Public Health (MVPH) of Freie Universität Berlin and Chiang Mai University, Thailand

[2] Veterinary Public Health Centre for Asia Pacific (VPHCAP) , Faculty of Veterinary Medicine, Chiang Mai University, Thailand

[3] Department of Veterinary Biosciences and Veterinary Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Thailand

[4] Department of Veterinary Public Health, Faculty of Veterinary Medicine, Hanoi University of Agriculture, Vietnam

[5] Institute of Food Hygiene, Faculty of Veterinary Medicine, Freie Universität Berlin, Germany

*Corresponding author; Email: thutra_ty48c@yahoo.com.vn

ABSTRACT *Vibrio* spp. are natural inhabitants of the aquatic environment. Among the members of the genus, twelve species have been reported to be pathogenic to humans and can cause foodborne infections. The aim of the recent study was to investigate the prevalence and the antimicrobial resistance patterns of *Vibrio* spp. isolated from retail shrimps in Hanoi, Vietnam. A total of 202 shrimp samples were collected from retail markets located in ten urban districts of Hanoi. Among those, 201 (99.5%) samples were positive for *Vibrio* spp. The most common species detected was *V. parahaemolyticus* (95.5%), followed by *V. alginolyticus* (56.4%), *V. cholerae* (2%) and *V. vulnificus* (1.5%). Multiple *Vibrio* spp. were found in 114 (56.4%) samples. In total, 195 *V. parahaemolyticus* isolates, four *V. cholerae* isolates and three *V. vulnificus* isolates, which are *Vibrio* species associated with foodborne infections, were tested for resistance to eight antimicrobial drugs using the disc diffusion method. *V. parahaemolyticus* isolates showed a high rate of resistance against ampicillin (87.2%), while a moderate rate was observed for sulfamethoxazole/ trimethoprim (18.5%) and intermediate resistance towards tetracycline (24.6%). Low resistance rates (0.5%) were recorded against both ciprofloxacin and cephalothin. Only one *V. cholera* isolate with resistance to ampicillin and two *V. cholerae* isolates to sulfamethoxazole/trimethoprim were found. All of the *V. vulnificus* isolates (n=3) were susceptible to the eight antimicrobial agents tested. Among the three *Vibrio* species, multi-resistance was found only in *V. parahaemolyticus* (16.9%). The result of this study indicates the high prevalence of *Vibrio* spp. in shrimp resistant to likewise ampicillin and sulfamethoxazole/ trimethoprim.

KEYWORDS: *Vibrio, prevalence, antimicrobial resistance*

INTRODUCTION

Vibrio is distributed worldwide in aquatic environment, including sea water, fresh water and brackish water. Twelve species of the genus *Vibrio* have been recognized to be pathogenic to humans(7). Among those, *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* are well known as the most important cause of foodborne infections, usually associated with consumption of raw or

undercooked seafood (16, 21). Recent studies demonstrated the presence of *Vibrio* in seafood (10, 18, 25, 26). Besides, the individual and multiple antimicrobial resistance of *Vibrio* spp. were also identified(2, 20).

Within the last decade, shrimp aquaculture has developed rapidly in Vietnam. Until today more than 3390 shrimp farms are registered (12). Black tiger shrimp (*Penaeus monodon*) and Pacific white shrimp (*Litopenaeus vannamei*) are the main species grown along the coast. Both species are used for domestic consumption as well as export purpose. Owing to the rapid enlargement of intensive aquaculture with inappropriate planning, a large amount of antibiotics is extensively used in shrimp production (8, 23). However, it was shown that the use of antibiotic drugs may lead to the development of antimicrobial resistances in pathogenic bacteria which can cause negative impact to aquatic animals as well as human health (15).

Up to now, less attention has been paid regarding pathogenic *Vibrio* species in Vietnam so far. There is limited information on the risk of *Vibrio* infection of human regarding consumption of seafood including shrimp. This study was conducted to determine the prevalence and to identify antimicrobial resistant patterns of *Vibrio* spp. isolated from retail shrimp.

MATERIALS AND METHODS

Sample collection

A total of 202 shrimp samples were collected from 52 retail markets in ten urban districts of Hanoi between January and April2013.

Bacterial isolation and identification

Tests were carried out according to the international standards of ISO/TS 21872-1:2007 (E) for *V. cholerae* and *V. parahaemolyticus* and ISO/TS 21872-2:2007 (E) for *Vibrio* spp. other than *V. cholerae* and *V. parahaemolyticus* with some modifications. A 25g-aliquot of the sample was mixed well with 225g of alkaline peptone water (APW, Merck®) with 2% NaCl and incubated at 37°C for 24 hours. After enrichment, a loop of the incubated medium was streaked onto Thiosulfate Citrate Bile Sucrose (TCBS, Difco®) agar and incubated at 37°C for 24 hours. Five typical colonies were selected and streaked on Lysogeny broth agar (LB agar) containing 1% NaCl and incubated at 37°C for 24 hours for biochemical confirmation. If there were less than five suspected colonies on the plate, all of these colonies were sub-cultured. All isolates colonies were screened by oxidase test, gram staining and motility test.

Species confirmation by multiplex Polymerase Chain Reaction (mPCR) method

Boiling method was used for DNA extraction. A multiplex PCR was conducted based on the method described by Bauer and Rorvik (3)and Di Pinto *et al.*(9). Table 1 shows primers used for mPCR. DNA templates of *V. parahaemolyticus*, *V. alginolyticus*, *V. cholerae* and *V. vulnificus* strain isolates from FreieUniversitätBerlin were used as positive controls; sterilized de-ionized water used as negative control. DNA amplification was performed in a thermocycler (Veriti™ 96-Well Fast Thermal Cycler, Singapore) using initial denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 92°C for 40 seconds, annealing at 62°C for 60 seconds, elongation at 72°C for 90 seconds and a final extension at 72°C for 7 minutes.

Antimicrobial resistance tests

V. parahaemolyticus, *V. cholerae* and *V. vulnificus* isolates were tested for resistances using the disc diffusion method according to guidelines set by the Clinical and Laboratory Standards Institute (CLSI) (5, 6). *E. coli* ATCC 25922 was used for quality control. Eight antimicrobial drugs (Oxoid®) used included ampicillin (10µg), chloramphenicol (30µg), ciprofloxacin (5µg), gentamicin (10µg), imipenem (10µg), cefalothin (30µg), sulfamethoxazole/ trimethoprim (23.75µg/ 1.25µg) and tetracycline (30µg).

RESULTS

Prevalence of Vibrio spp. *from retail shrimps*

The study revealed that 201 (99.5%) of all samples contained at least one detectable *Vibrio* species. *V. parahaemolyticus* was the most common prevalent (96.5%), followed by *V. alginolyticus* (56.4%), *V. cholerae* (2%) and

Table 1: Sequences of primers for the mPCR.

Target species	Primer	Sequence (5′ to 3′)	Amplicon size (bp)	References
	UtoxF	GAS TTT GTT TGG CGY GAR CAA GGT T		(3)
V. parahaemolyticus	VptoxR	GGT TCA ACG ATT GCG TCA GAA G	297	(3)
V. cholerae	VctoxR	GGT TAG CAA CGA TGC GTA AG	640	(3)
V. vulnificus	VvtoxR	AAC GGA ACT TAG ACT CCG AC	435	(3)
V. alginolyticus	VA-F	CGA GTA CAG TCA CTT GAA AGC C	737	(9)
	VA-R	CAC AAC AGA ACT CGC GTT ACC		

V. vulnificus (1.5%) (Figure1). Multiple *Vibrio* spp. were found in 114 (56.4%) samples (Table 2). Among those, *V. parahaemolyticus* and *V. alginolyticus* dominated in 107 samples (93.8%).
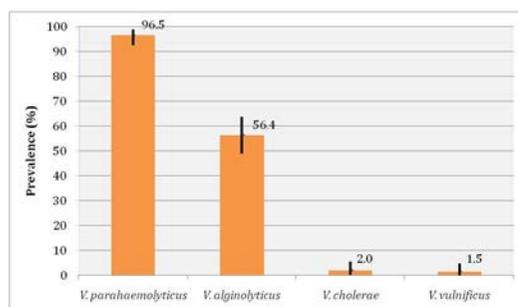
Figure 1: Prevalence of *Vibrio* spp. in shrimp from retail markets in Hanoi.

Table 2: Prevalence of multiple *Vibrio* spp. in retail shrimp.

Range of *Vibrio*	Prevalence (%)	Confidence Interval (95%)
One *Vibrio* spp.	43.1 (87/202)	36.32 - 50.00
Multiple *Vibrio* spp.	56.4 (114/202)	49.50 - 63.18

Antibiotic resistance pattern of Vibrio spp. isolated from shrimp

From each positive sample, one isolate of *V. parahaemolyticus*, *V. cholerae* and *V. vulnificus* was selected for antimicrobial resistance test. Therefore, 195 isolates of *V. parahaemolyticus*, four *V. cholerae* isolates and three *V. vulnificus* isolates were tested against eight antimicrobial agents. Only one

V. cholera isolate was resistant to ampicillin and two *V. cholerae* isolates to sulfamethoxazole/ trimethoprim. All of the *V. vulnificus* isolates (n=3) were susceptible to all eight antimicrobial agents tested.

Table 3: Distribution of antimicrobial susceptibility of *V. parahaemolyticus* isolates (n = 195).

Antimicrobial agent	% Resistant (n)	% Intermediate (n)	% Susceptible (n)
AMP 10	87.2 (170)	9.7 (19)	3.1 (6)
C 30	0 (0)	0 (0)	100 (195)
CIP 5	0.5 (1)	1.5 (3)	97.9 (191)
CN 10	0 (0)	0 (0)	100 (195)
IPM 10	0 (0)	0 (0)	100 (195)
KF 30	0.5 (1)	2.6 (5)	96.9 (189)
TE 30	1.5 (3)	24.6 (48)	73.8 (144)
SXT 25	18.5 (36)	4.6 (9)	76.9 (150)

Note: AMP 10 = ampicillin, C 30 = chloramphenicol, CIP 5 = ciprofloxacin, CN 10 = gentamicin, IPM 10 = imipenem, KF 30 = cefalothin, SXT 25 = sulfamethoxazole/trimethoprim

Table 3 demonstrates antimicrobial susceptibility profiles of *V. parahaemolyticus* isolates. The highest rate of resistancewas observed towards ampicillin (87.2%), while a moderate rate was recorded for sulfamethoxazole/ trimethoprim (18.5%) and an intermediate resistance towards tetracycline (24.6%). Resistances against other antimicrobial drugs were found to be low (0.5%) towards ciprofloxacin and cefalothin. None of the *V. parahaemolyticus* isolates was resistant to chloramphenicol, gentamicin and imipenem.

Among the three *Vibrio* species, multi-resistance was found only in *V. parahaemolyticus* (16.9%) (Table 4). Strains owning multiple antibiotic resistances were found to be resistant towards two or three antibiotics tested. Five different profiles were recorded among multi-resistant isolates: AMP+KF, AMP+TE, AMP+SXT, AMP+CIP+SXT and AMP+TE+SXT.

Table 4: Antimicrobial resistance patterns of *V. parahaemolyticus* isolates.

Resistances	Patterns	No. of isolates	%
One (antimicrobial drug)	AMP	137	70.3
	SXT	6	3.1
Two (antimicrobial drugs)	AMP+KF	1	0.5
	AMP+TE	2	1.0
	AMP+SXT	28	14.4
Three (antimicrobial drugs)	AMP+CIP+SXT	1	0.5
	AMP+TE+SXT	1	0.5

DISCUSSION

Vibrio species occur naturally in aquatic environments. Seafood harvested from contaminated water or inappropriately preserved after harvesting is known to play an important role in human infections by *Vibrio* spp. (1). Therefore, the presence of these organisms in raw seafood including shrimp could be expected. This study found that 99.5% (201/202) of the shrimp samples taken from retail markets in Hanoi were positive for *Vibrio* spp. The data corroborates findings that *Vibrio* spp. is the most common zoonotic bacteria found in shrimp.

V. parahaemolyticus was dominant in shrimp samples (96.5%), this result corresponds to a study on the prevalence of *Vibrio* spp. in pre-harvest shrimp of Sri Lanka (14). However, other studies revealed different results. Instead of *V. parahaemolyticus*, the most frequently isolated species was *V. alginolyticus*(1, 24). In contrast to the high prevalence of *V. parahaemolyticus* and *V. alginolyticus*, the present study observed low percentages of *V. cholerae* (2%) and *V. vulnificus* (1.5%). It was shown that the occurrence of *V. cholerae* was associated with warm water temperature and zooplankton blooms(11). This study was conducted during winter and spring in the North of Vietnam, hence the low prevalence of *V. cholerae* was observed.

Even if *V. parahaemolyticus* was the most common found in samples, among *V. parahaemolyticus* only a small percentage of strains are pathogenic and can cause acute gastroenteritis, those produces toxins including thermostable direct haemolysin (TDH) and/or the thermostable direct related haemolysin (TRH), encoded by the *tdh* and *trh* genes, respectively (13, 17). This study did not detect virulent genes of *V. parahaemolyticus* isolates. However, even non toxin encoding strains were reported causing food associated disease(19).

V. vulnificus is also an important *Vibrio* species that can cause mild to severe gastroenteritis associated with the consumption of contaminated food or exposure to the contaminated water (4), as well as causing wound infections and a serious septicemia leading to death frequently. Therefore, despite a very low prevalence in this study (1.5%), the presence of *V. vulnificus* should be considered as public health concern.

Regarding antimicrobial resistance tests, the present study indicated that 171 isolates (84.7%) and 38 isolates (18.8%) of *Vibrio* spp. were found to be resistant to ampicillin and sulfamethoxazole/ trimethoprim, respectively. By contrast, a low degree of resistance to tetracycline, ciprofloxacin and cefalothin was observed. Results are in agreement with other authors (2, 20, 22) who indicated high resistance of *Vibrio* spp. isolates from shrimp to ampicillin. Multiple resistance was observed in 16.3% (33/202) of *Vibrio* isolates, compared to 29% in a study by Rebocas *et al.*(20).

CONCLUSION

The present study, which demonstrated high prevalence of *Vibrio* spp. as well as individual and multi-resistance to antimicrobial drugs tested, has shown the evidence of hazard potential of shrimp containing potentially pathogenic *Vibrio* spp.

Consuming raw or undercook shrimp should be avoided to reduce the risk of *Vibrio* infection. Besides, the use of antibiotics in shrimp culture should also be of concern.

ACKNOWLEDGEMENT

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Effect of Dengue Virus Infection of Long-tailed Macaque (*Macaca fascicularis*) in Kosumpee Forest Park, Maha Sarakham Province

Captain Gerdsuwan[1] Chonruetei Sukma[1] Susadee Khemton[1] Natapol Pumipuntu[2,*] Pailin Jinagool[3] Worapol Aengwanich[4] Pornpit Vaisusuk[5]

[1] *Faculty of Veterinary Sciences, Mahasarakham University, Mahasarakham 44000, Thailand*
[2] *Department of Veterinary Public Health, Faculty of Veterinary Sciences, Mahasarakham University, Mahasarakham, Thailand*
[3] *Department of Veterinary Clinical Science, Faculty of Veterinary Sciences, Mahasarakham University, Mahasarakham, Thailand*
[4] *Department of Physiology, Faculty of Veterinary Sciences, Mahasarakham University, Mahasarakham, Thailand*
[5] *Veterinary Animal Hospital, Faculty of Veterinary Sciences, Mahasarakham University, Mahasarakham, Thailand*
Corresponding author; Email: film.natapol@gmail.com

ABSTRACT The study of hematological values and effect of dengue virus infection in Long-tailed Macaques (*Macaca fascicularis*) were determined in Long-tailed Macaques at Kosumpee Forest Park, MahaSarakham province. The blood samples were collected from cephalic vein and femoral vein of 30 macaques. Dengue virus antibodies detected by using a commercial test kit, the results of Dengue virus infection was found 6 infected macaques (20% of total samples) and 24 non-infected macaques (80% of total samples) show the incidence of Dengue virus infection in Long-tailed Macaques at Kosumpee Forest Park was occurred 1/5 of macaque population. Hematological values (mean ± SD) of non-infected macaques, founded WBC count 20438.89 ± 5703.88 x 10^3cell/µl, Hemoglobin 13.04 ± 1.21 g/dL, Hemetocrit 39.67 ± 3.71%, Neutrophil 29.67 ± 13.76%, Lymphocyte 60.45 ± 14.29%, Monocyte 4 ± 2.44%, Eosinophil 5.89 ± 3.14 %, Platelet 300833.34 ± 103071.22 cell/µl, RBC count 5.05 ± 0.39 x 10^6cell/µl, MCV 78.19 ± 2.12 fL, MCH 24.60 ± 5.02 pg, MCHC 32.16 ± 1.06 g/dL, was differenced in white blood cell count between infected and non-infected macaques (P<0.05). The results of this study can be used as a guideline for the prevention of disease from animals to humans.

KEYWORDS: *Dengue virus, Hematology, Long-tailed Macaques, Kosumpee Forest Park*

INTRODUCTION

Based on the most recent public health problems worldwide, there are many infectious diseases from animals to human especially from non-human primates, including Shigellosis, Tuberculosis, Ebola virus, Marburg virus, Herpes B virus, Amoebiasis and Giardiasis. Because of their close phylogenetic relations to humans, non-human primates have an important role in research into human-animal disease.

Long-tailed macaques (*Macaca fascicularis*) have the widespread geographical distribution among primate population. They are distributed a wide area of Southeast Asia (1). They inhabit a wide variety of habitats, including primary lowland rainforests, disturbed and secondary

rainforests, and riverine and coastal forests of nipa palm and mangrove (2).

In Thailand, long-tailed macaques are the most frequently observed species (3). Presently humans have invaded and disturbed the natural habitats of primates by forest destruction (2). As the alternative habitat forced long-tailed macaques to share habitat and adapted to living with humans, was found in many places in Thailand, including Kosumpee Forest Park in MahaSarakham province.

Dengue virus (DENV) is one of the most important mosquito-borne diseases (4) that transmit to humans through the bites of infective *Aedes* mosquitoes (4), which are significant and seriously affected public health problems in Southeast Asia. The prevalence of DENV has substantially grown in every year. The annual average number of DENV cases in MahaSarakham province reported by Bureau of Epidemiology, Thailand found 265 cases in 2012s, KosumPhisai district, the third case number, where is the location of Kosumpee Forest Park has 44 cases.

MATERIALS AND METHODS
Animals / Sample collection

The study of dengue infection and hematological values were determined in long-tailed macaques (*Macaca fascicularis*)at Kosumpee Forest Park, MahaSarakham province. Thirty macaques were temporarily caught in soft mesh cage (4x4x1.5 m). The blood samples were collected from cephalic vein and femoral vein.

Laboratory Examination

Dengue diagnostic using Dengue IgG/IgM Combo Rapid Test (CTK Biotech Inc., San Diego) indicated the detection of anti-dengue virus antibodies into dengue infection. Apply 1 drop of whole blood (about 40-50 μL) into the sample. Then add 1 drop (about 35-50 μL) of sample diluents immediately. Positive result, in addition to the presence of C band, if only T1 band is developed, indicates for the presence of IgG anti-dengue virus, in addition to the presence of the C band, if only T2 band is developed, the test indicates for the

presence of IgM anti-degue virus, in addition to the presence of C band, both T1 and T2 bands are developed, indicates for the presence of IgG and IgM anti-dengue virus. Negative result, if only the C band present, the absence of the burgundy color in the both T bands (T1 and T2) indicates that no anti-dengue virus antibodies are detected.

Hematological Examination

The blood samples were collected from cephalic vein and femoral vein of 1-2 ml into EDTA tube and microcentrifuge tube. The number of red blood cell and white blood cell were counted by microscope. Hematocrit centrifugation and Cyanmethemoglobin are using for determined hematocrit values and hemoglobin values, respectively. Determination of red blood cell indices, including mean corpuscular volume (MCV), mean corpuscular heamoglobin (MCH) and mean corpuscular hemoglobin in concentration (MCHC), by calculating the number of red blood cells, packed cell volume and hemoglobin concentrations.

Data analysis

The data were analyzed by T-test and descriptive statistics. The results of dengue infection were analyzed by percentage. One sample T-test was performed to compare hematological values.

RESULTS

The detection of dengue virus (DENV)-specific immunoglobulin into dengue infection is 6 of 30 samples (20 %) and 24 of 30 samples (80%) without anti-DENV immunoglobulin. Showed the incidence of dengue infection is 1 in 5.

Hematological values (Mean ± SD) of the two groups, one sample T-test are summarized in Table 1. *The results was differenced in white blood cell*, hemoglobin, *hematocrit,* neutrophil, lymphocyte, *red blood cell*, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) ($p < 0.05$).

Hematological values in long-tailed macaque *(Macaca fascicularis)* compared the anti-DENV immunoglobulin non-evident samples at Kosumpee Forest Park with the hematological values of macaque at Pranakornkhiri Historical Park, Phetchaburi province (5), found difference between hemoglobin, lymphocyte and eosinophil are lower and neutrophil is higher than the macaque at Kosumpee Forest Park.

the incidence of dengue infection is 1 in 5, that is based on the hypothesis. Kosumpee Forest Park has the Chi River flows through, flooding every year during of July to September, that causing increased a proliferation of *Aedes aegypti*. Result in the spread of the disease in animals and people in the KosumPhisi district. The annual average summary of DENV cases reported by Bureau of Epidemiology, Thailand in MahaSarakham province has 42 patients of 100,000

Hematological values	Unit	Normal (n=18)	Detect antibody (n=4)	p-value
Red blood cell count (RBC)	$x10^6$ cell/μL	5.05±0.39	5.15±0.49	0.006
Hemoglobin	g/dL	13.04±1.21	12.97±1.24	0.002
Hematocrit	%	39.67±3.71	39.25±3.5	0.003
Mean corpuscular volume	fL	78.19±2.12	76.22±3.17	0.008
Mean corpuscular hemoglobin	pg	24.60±5.02	25.17±1.21	0.007
Mean corpuscular hemoglobin concentration	g/dL	32.16±1.06	32.37±0.82	0.002
White blood cell count (WBC)	$x10^3$ cell/μL	20438.89±5703.88	19550±5871.12	0.014
Neutrophil	%	29.67±13.76	31±8.87	0.014
Lymphocyte	%	60.45±14.29	58.75±8.38	0.009
Monocyte	%	4±2.44	5.75±2.99	0.113
Eosinophil	%	5.89±3.14	4.5±3.51	0.085
Platelet	μL	300833.34±103071.22	387250±84543.38	0.08

n = Number of infected or uninfected macaques.

Table 1 Shows a comparison of hematological values in long-tailed macaque *(Macaca fascicularis)* between the anti-DENV immunoglobulin evident and non-evident samples at Kosumpee Forest Park, Maha Sarakham province (Mean ± SD).

The result of hematological values in long-tailed macaque *(Macaca fascicularis)* at Mauritius (6) was found that the number of mean corpuscular hemoglobin concentration (MCHC), white blood cell and eosinophil are lower, otherwise red blood cell count, hematocrit are higher than the macaque at Kosumpee Forest Park, MahaSarakham province.

DISCUSSION

The DENV exposure of long-tailed macaque *(Macaca fascicularis)* at Kosumpee Forest Park, MahaSarakham province, was found 20% of total samples (n = 30) showed

populations. Monthly summary of the 2011s, there is the most patients in July (3). Kantharawichai, Muang and Kosum district are the maximum number of patients. Moreover, Dengue virus in the family flaviviridae has 4 serotype (DEN-I, DEN-2, DEN-3 and DEN-4) and the antigens share some part that can be detected by the antibody used in commercial test kit. So they can be a relationship between the DENV positive macaques and the DENV human cases in this area.

The white blood cell count between infected and non-infected macaques were differenced significantly (P<0.05). The infected macaques has lower white blood cell

level, because of hepatocytes, neuron cells, dendritic cells, endothelial cells, monocyte/ macrophage, B cells and T cells are the target cell of Dengue virus. This study, the infected has higher red blood cell level more than non-infected macaques, because samples were collected after improve or secondary infection. The platelet value is normal, because the samples were infected longer than 7 days. The report showed platelet value is low after infected at 7 days (7).

The resource of dengue virus infection in humans showed the hematological values that leukocytosis is common, and moderate

humans. Conversely, monocytosis, eosinopenia and thrombocytosis are difference, that may caused by species and duration of infection.

The comparison of hematological values of long-tailed macaque in Kosumpee Forest Park, MahaSarakham province, Pranakornkhiri Historical Park, Phetchaburi province (5) and Mauritius (6), found that hemoglobin, white blood cell, neutrophil, eosinophil and platelets were differenced significantly (P<0.05). Even, the same monkey species (*Macaca fasicularis*) but the difference habitats and environment factors, including water, food, climate,

Hematological values	Unit	Normal (n = 18)	Range	Detect antibody (n = 4)	Macaque 1	Macaque 2
Red blood cell count (RBC)	x10⁶ cell/µL	5.05±0.39	4.66 - 5.44	5.15±0.49	387±84.54	7.03±0.24
Hemoglobin	g/dL	13.04±1.21	11.83 - 14.25	12.97±1.24	11.49±1.02	14±0.42
Hematocrit	%	39.67±3.71	35.96 - 43.38	39.25±3.5	38.55±3.07	49.75±0.63
Mean corpuscular volume	fL	78.19±2.12	76.07 - 80.31	76.22±3.17	-	71±1.41
Mean corpuscular hemoglobin	pg	24.60±5.02	19.58 - 29.62	25.17±1.21	-	19.95±0.07
Mean corpuscular hemoglobin concentration	g/dL	32.16±1.06	31.1 - 33.22	32.37±0.82	-	28.15±0.63
White blood cell count (WBC)	x10³cell/µL	20.43±5.7	14.73 – 26.13	19.55±5.87	16.81±4.83	8.5±2.12
Neutrophil	%	29.67±13.76	15.91 - 43.43	31±8.87	80.81±8.60	25.85±0.07
Lymphocyte	%	60.45±14.29	46.16 - 74.74	58.75±8.38	15.93±7.52	66.45±2.61
Monocyte	%	4±2.44	1.56 - 6.44	5.75±2.99	2.48±1.89	5.45±1.62
Eosinophil	%	5.89±3.14	2.75 - 9.03	4.5±3.51	0.74±1.30	1.05±0.63
Platelet	µL	300.83±103.07	197.76 - 403.9	387±84.54	400.01±124.69	371.5±212.13

M1 = Pranakornkhiri Historical Park, Phetchaburi province (Prapasanobol, 2012)

M2 = Mauritius (Ugo et al., 2009)

Table 2 Shows a comparison of hematological values in long-tailed macaque (*Macaca fasicularis*) between the anti-DENV immunoglobulin evident and non-evident samples at Kosumpee Forest Park, Maha Sarakham province, including the hematological values of long-tailed macaque at Pranakornkhiri Historical Park, Phetchaburi province (Prapasanobol, 2012) and Mauritius (Ugo et al., 2009)

thrombocytopenia is occasionally observed (8). The most indicated values are level of neutrophil and lymphocytes that become lower and higher at 3th or 4th date of infection, respectively. Platelets are decreased approximately 10,000 – 50,000 /mm at 3th or 4th date of the infection. Otherwise, hematocrit (Hct) increased slowly until the day before shock fever (8).

Some hematological values of non-evident anti-DENV immunoglobulin group were differenced in leukocytes significantly (P<0.05). Leukocytosis and leukopenia were found to be similar to dengue infection in

topography and endemic pathogens affected the growth of the macaques.

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Behaviour of Malaria Prevention in the Five Highest Case Incidences of Malaria Provinces in Indonesia

Dewi Susanna[1] Tris Eryando[2]

[1] *Department of Environmental Health, Faculty of Public Health, Universitas Indonesia*
[2] *Departments of Population and Biostatistics, Faculty of Public Health, Universitas Indonesia*
Corresponding author; Email: dsusanna2@yahoo.com

ABSTRACT The big five provinces with high case incidence of malaria in Indonesia were Papua, Barat, Nusa Tenggara Timur (NTT), Maluku Utara, and Bangka-Belitung. This study objective was to know the differences in behaviour to prevent the transmission of malaria in the 5 highest provinces of malaria incidence in Indonesia. This study used the secondary data from National Basic Health Research conducted by Ministry of Health Republic of Indonesia in 33 provinces in Indonesia. Household (HH) samples were selected based on Block Census (BC). There were 2,798 BC interviewed. All eligible member of selected HH were interviewed as a sample, they were 251,388 respondents. The malaria case determined by interview using questionnaire which confirmed by the medical examination result. Data were analysed using non parametric test- chi square test for all variables using 95% significance level (alpha = 0.05) to test the average of the highest 5, others (28 provinces) and Indonesia (33 provinces-weighted) for all variables. Among 33 provinces in Indonesia, they were 5 provinces have a highest incidence of malaria. The average malaria incidence in Indonesia was 22.9‰, ranged from 3.4‰ to 261.5‰. The five highest provinces of malaria incidence per 100,00 were 2.62 in Papua; 2.54 in Barat Papua; 1.18 ‰ in NTT; 1.03; 1.03in North Maluku, and in Bangka-Belitung. The incidence of malaria per 100,000 in the five highest provinces was statistically different (p value < 0.05). Almost all variables (sleeping under bed net, repellent, burned/electric insecticide, insecticide spray, take a pill/drug for prevention or prophylaxis, and other methods of prevention) measured showed that in the 5 provinces with highest incidence and others (28provinces) and Indonesia (weighted) were significantly different (p value < 0.05), except using net for window/ventilator (p value > 0.05).

KEYWORDS: *malaria, province, prevention, behaviour, high case incidence*

INTRODUCTION

Indonesia consists of 13,466 islands (1) with relatively different geographic conditions and divided into 33 provinces.49.70% of population lived in urban areas (2), and 57.50% of the population lived in Java Island (3). Papua and Papua Barat provinces are in big island in the east part of Indonesia, while Nusa Tenggara Timur and Maluku Utara provinces are also in the east part of Indonesia are consist of a couple of relatively smaller islands, while Bangka-Belitung province is in

the small islands in west part of Indonesia. The Anopheles vector inhabitants in those big and small islands were also found differently, even though some species found in all areas (4).

Decreasing mortality and morbidity of malaria is one of the Millennium Development Goals (MGDs) targets in 2015. The survey of National Basic Health Research conducted by Ministry of Health Republic of Indonesia (2010) found that the number of malaria in Indonesia reaches 417 thousand cases (2012), with nearly three-quarters of the cases were from Papua, Barat Papua and Timur Nusa Tenggara (ENT).(5)

Based on the differences condition of the provinces, it was assumed that people behaviour and programs run to eradicate the death causes of and case of malaria in each province could be different. It was important to understand the malaria prevention behaviour of the people in each province. The programs introduced by the ministry of health weremosquito net for bed and ventilation, vector control, case finding, diagnosis and malaria treatment (4).

One of malaria eradication method was by cutting the mode of malaria transmission by avoiding the mosquito bite. It is already confirmed that the number of Anopheles are 25 type of Anopheles spread around area of Indonesia(4).Some of the methods for avoiding mosquito bite were using insecticide mosquitos net while sleeping, use fumigant coil, repellent, use long sleeves, take a prophylaxis pill, and apply mosquito window/ventilation net(5). These methods were very important to understand especially in the areas with high case of malaria incidence. This study objective was to know the differences in population behavior to prevent the transmission of malaria in the 5 highest provinces of malaria incidence in Indonesia, due to the evidence that those areas still highest malaria incidence.

MATERIALS AND METHODS

This study used the secondary data from Basic Health Research or Riset Kesehatan Dasar (RISKESDAS) conducted by National Institute of Health Research and Development, Ministry of Health Republic of Indonesia in 33 provinces in Indonesia(5). This study used the secondary data from National Basic Health Research conducted by Ministry of Health Republic of Indonesia in 33 provinces in Indonesia. Household (HH) samples were selected based on Block Census (BC). There were 2,798 BC interviewed. All eligible member of selected HH were interviewed as a sample, they were 251,388 respondents. The malaria case determined by interview using questionnaire which confirmed by the medical examination result. The variables to measure the behaviour of prevention were: sleeping under bed net, using net for window/ ventilator, repellent, mosquito coil/electric, insecticide spray, takes a pill/drug for prevention (prophylaxis), and other methods of prevention. Interview addressed to people above 15 years old. The data of average of the highest 5 provinces, others (28 provinces) and Indonesia (33 provinces-weighted) analysed using non parametric test- chi square test for all variables using 95% significance level (alpha = 0.05). The secondary data also provided by other resources that were National Statistic Board, National Geography-Indonesia, Indonesia data.co.id, and Ministry of Health.

RESULTS

Table 1 showed the average incidence of malaria (per 100,000) in the highest 5 provinces had a quite high compare with other provinces and Indonesian in average (weighted) as well.

Table 1 showed that the proportion of incidence of malaria in the five highest provinces was statistically different (p value < 0.05). Almost all variables (sleeping under bed net, repellent, burned/electric insecticide, insecticide spray, take a pill/drug for prevention or prophylaxis, and other methods of prevention) measured showed that in the 5 provinces with highest incidence and others (28 provinces) and Indonesia (weighted) were significantly different (p value < 0.05), except using net for window/ventilator (p value > 0.05).

Table 1: The proportion of behaviour prevention of malaria in the 5 Highest Case Incidence of Malaria, Indonesia 2010.

Name of Province	Behaviour in malaria prevention (subject > 15 years old)							Incidence (per 100.000)
	sleeping under bednet (%)	Mosquito coil/electric (%)	net for window/ ventilator (%)	Repellent (%)	insecticide spray (%)	take a pill for prophilaxis (%)	Others (%)	
Papua	50.3	42.1	15.9	12.6	12.6	11.2	8.3	2.62
Papua Barat	54.1	41.1	23.7	15.8	24.3	8.3	12.3	2,53
Nusa Tenggara Timur (NTT)	58.7	30.0	8.5	12.1	10.6	6.1	9.3	1.18
Maluku Utara	36.6	49.7	4.9	7.0	10.0	3.2	7.7	1.03
Bangka-Belitung	38.6	73.1	14.8	14.5	24.4	5.5	15.9	0.92
p value	*< 0.05*	*< 0.05*	*0.05*	*< 0.05*	*< 0.05*	*< 0.05*	*< 0.05*	*< 0.05*
Average (5 provinces)	47.7	47.2	13.6	9.9	16.4	5.6	10.7	1.66
Average (28 provinces)	42.28	61.63	15.24	19.28	20.26	5.67	12.36	0.30
Indonesia (weighted)	31.9	57.6	13.6	24.7	20.0	4.7	13.2	0.23
p value	*< 0.05*	*< 0.05*	*0.33*	*< 0.05*	*< 0.05*	*< 0.05*	*< 0.05*	*< 0.05*

DISCUSSION

This study was a secondary analysis in national level, not using individual basis data. The proportion behaviours in preventing malaria transmission in all provinces were different. It means that each province may have different programme or intervention in the community due to the variety of socio demography factors. It is known that population density was also quite different one to another (6). The incidence of malaria in those 5 provinces may describe the social demographic such as population density, the type of Anopheles as well (4). Maluku Utara and Bangka-Belitung provinces only have one vector, while Papua, Papua Barat and NNT has more than one vector (4).

Sleeping under bed net and use of mosquito coil/electric were the most behaviour in practice compare with other preventions, whereas the proportion in take a medicine for prophylaxis put the lowest proportion in prevention. Variables which can be used to help reduce or prevent malaria include behaviours that reduce night-time activity outdoors, wearing covering clothing, using repellents, bed nets, and mosquito-proofing homes (7). Use of repellents, mosquito coils and sleeping arrangements were significant in some of other studies. Erdinal (2006), also mentioned that using net or screen, bed net, and mosquito coils associated with malaria, but using repellent

did not (8), besides the use of bed nets, pattern of outdoors activities, especially at night are important factors (7).

Almost 50 % respondents sleep under bed net and use mosquito coils/electric. Bed nets are considered essential by WHO in control program because they provide protection from mosquitos (9), and may have a significant effect in reducing the incidence of malaria (10). A total of 1.4 million nets distributed among the five provinces in the eastern region of Indonesia that still has a fairly high incidence of malaria. Those five provinces are Papua, Papua Barat, Nusa Tenggara Timur, Maluku and Maluku Utara (11).

Long lasting insecticidal nets (LLIN) is one effective way to prevent malaria (12). Permethrin treated LLIN is one type of LLIN which is recommended by WHO. In 2006, UNICEF, in collaboration with the Ministry of Health introduced the LLIN (Olyset®, Sumitomo Co. Japan) in Indonesia. In Bangka regency, there were 60,000 LLIN were distributed to the public made from polyethylene and contain the insecticide permethrin (11).

Several studies have shown that these types of LLIN requiring heat assisted regeneration after washing to enhance the biological activity of insecticide that contained in the LLIN fibers (13). Ineffective usage of ITNs and knowledge gaps on malaria transmission, signs and symptoms, prevention

and control predisposed communities in the district to malaria epidemics (13). To increase the society knowledge, they need explanation of the purpose of permethrin insecticide-treated nets distribution, how to use permethrin insecticide-treated nets, how to wash and who are the priorities of family to use permethrin insecticide-treated nets. In addition it should also be socialized about mosquito net heating (heat-assisted regeneration) in order to restore the effectiveness of the insecticide on the nets. (14). The use of insecticide-treated nets (ITNs) influenced by socioeconomic factors (14). Ineffective usage of ITNs and knowledge gaps on malaria transmission, signs and symptoms, prevention and control predisposed communities in the district to malaria epidemics. It is important that health education packages are developed to address the identified knowledge gaps (13).

Net or screen roles as an entry point of mosquito to come into the house. House construction has an effect on malaria infection. The general relationship is that malaria incidence is higher in houses of flimsy construction which permit the entry of mosquitos (16), so improving house construction using net or screen should promoted by government (15). At the village level, in Central Java, there was no significant association between house type and malaria, though screen was associated with a reduced incidence of malaria (17).

From the finding of this research, it suggested to raise the understanding of illness at house hold level which can enhance appropriate home management of malaria especially their behaviour in preventing the transmission and more effective use of insecticide-treated nets (ITNs). The community advised to have livestock's in their house, and this variable should be collected by survey together with any other factors contributing in malaria transmission.

Indonesia is the big area, consists of many island and the population lives in rural areas around 51.30% (17). Malaria mostly exists in rural areas, it is necessary to focus the issue in the rural area. In this case in the rural areas

need to be promote the repellent, since they activity during the night is in an open space not like in the urban areas. Bed nets usually provided for rural area, because in urban area malaria is not common.

Indonesia experienced in a high proportion of mosquito coil in average, so it is still an important factors to be promoted all over the country.

CONCLUSION

The incidence of malaria in the five highest provinces was statistically different with other provinces and Indonesia (weighted) and all variables indicating the behaviour in prevention (sleeping under bed net, repellent, burned/electric insecticide, insecticide spray, take a pill/drug for prevention or prophylaxis, and other methods of prevention) were also different except the using net for window/ventilator. It is necessary to differentiate the data into urban and rural areas, since malaria mostly exists in the rural areas.

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Hygienic Practices, Knowledge and Perception on Food Safety and Quality Assurance Systems in Poultry Slaughterhouses and Slaughter Poultry Market in Yogyakarta, Indonesia

Bongkot Phumkrachai[1,*] Hathaichanok Vasasiri[1] Parinya Samart[1] Fonthip Kongboonkeaw[2]
Fred Unger[8] Wayan T. Artama[4] DyahAyu Widiasih[5] Duangporn Pichpol[6] Tongkorn Meeyam[7]

[1] *Veterinary student, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand*

[2] *Graduate students, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand.*

[3] *International Livestock Research Institute, Nairobi, Kenya*

[4] *Department of Biochemistry, Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta, Indonesia*

[5] *Department of Veterinary Public Health, Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta, Indonesia*

[6] *Department of Veterinary Biosciences and Veterinary Public Health, Faculty of Veterinary Medcine, Chiang Mai university, Chiang Mai, Thailand*

[7] *Department of Veterinary Biosciences and Veterinary Public Health, Faculty of Veterinary Medcine, Chiang Mai university, Chiang Mai, Thailand*

[8] *International Livestock Research Institute (ILRI)*

Corresponding author; Email: ryukipu@gmail.com

ABSTRACT This study aims to identify perception, awareness and knowledge on food safety and quality assurance related to poultry slaughterhouses and slaughter poultry market places in Yogyakarta, Indonesia. Data collected from March to April 2013, from selected poultry slaughterhouses (n=9) and slaughter poultry market (n=1) located in 4 districts of Yogyakarta including Sleman, Bantul, Gunung Kidul and Kulon Progo. Questionnaires, in-depth interviews and checklists were applied to different stakeholders such as authorized veterinarians, owners or managers at slaughterhouses and slaughter poultry market. Results showed that knowledge on food safety and quality assurance is averagely good anyhow varying by slaughterhouses' owner or government. The current implemented standard for slaughterhouses for domestic consumption is well recognized, the understanding and knowledge are only well defined by officers. All stakeholders are aware of food safety, particularly related to meat quality and consumer health concerns. The standard for poultry slaughterhouses in Yogyakarta is current being developed. The understanding of hygienic practice and the demand of consumers for quality products may play an important role for standard perception, behavior change and increased food safety awareness in the community. The challenges towards standard implementation and its enforcement are the slaughtering practice and behavior, required slaughterhouse reformation, owners and workers knowledge, economic benefit of needed investment, and infrastructure with proper equipment.

KEYWORDS: *poultry slaughterhouse, knowledge, perception, food safety, quality assurance*

INTRODUCTION

Chicken meat is one of the most important animal protein products that people usually consumed. The increasing chicken meat demand is one of results from the rapid growth economics. The raising global economic and increasing world population make chicken meat consumption in 2009 was 6 kilogram per person per year (1).

These continue global economic and population growth makes large effects on increase chicken productivity to supply the incoming demand. The domestic chicken meat production of the Republic of Indonesia during 2000 – 2011 keeps growing up every year (2).Even the domestic productivity keeps growing (3), Indonesia still need imported products to balance demand – supply ration in the country. Because of the aspect of Indonesia's population will reach 262,569,000 persons from 2013 to 2021 (4), the demand still continuously increase. The main domestic chicken meat supply to Indonesia's domestic market comes from two sources. These two sources are poultry abattoirs in local area or surrounding region and local slaughter poultry market.

Because of the large domestic chicken meat consumption, food safety and quality assurance system have to be concern. Many food borne diseases such as Campylobacteriosis, Salmonellosis and *Escherichia coli* infection which have affect to public health. One of these organisms contaminations come from improper slaughtering processes at slaughterhouse (5, 6).

This study aims to identify the hygienic practices of the poultry slaughterhouses and slaughter poultry market in Yogyakarta, Indonesia. Perception, awareness and knowledge on food safety and quality assurance were also described.

MATERIALS AND METHODS

Sample population

Poultry slaughterhouses and slaughter poultry market were sampling by four districts of Yogyakarta. All slaughterhouses and slaughter poultry markets have to be operative slaughter poultry market and have its processing area. Both standard and non-standard in every scales of poultry slaughterhouses were also described.

Poultry abattoirs and slaughter poultry markets in each district were collect by convenient sampling. Most of slaughterhouses are backyard slaughterhouses and some consumers like to slaughter chicken at house. So, in each district at least 2 poultry slaughterhouses and 1 slaughter poultry market were visit.

In each district were collect data from poultry slaughterhouses' owner and/or managers and from local governmental veterinary staffs who role in local poultry slaughterhouses, slaughter poultry markets and poultry meat distribution. The data were collected from 9 slaughterhouses, 1 slaughter poultry market and 4 authorized veterinarian. The slaughter poultry markets in 3 districts except Sleman were no longer allowed to slaughter at market.

Data collections and tools

Data collection was collected on March to April, 2013 at selected poultry slaughterhouses and slaughter poultry market in 4 districts of Yogyakarta. The main issues that focus were hygienic practices, knowledge and perception on food safety and quality assurance systems.

Hygienic practices were collected by using questionnaires with slaughterhouses' owners and/or managers, checklist which follows by poultry abattoirs GMP and observation on poultry slaughterhouse places and slaughtering processes. Knowledge, perception and awareness to food safety and quality assurance system were collected by used questionnaires and In-depth interviews with poultry slaughterhouses' owners and managers. We used In-depth interview for knowledge, perception and implementation of poultry slaughterhouse's standard.

Data analysis

The questionnaires, checklist and observation on hygienic practices were analyzed in to descriptive statistic as percentile. Knowledge, perception, awareness

and standard implementation data were also analyzed into percentile.

RESULTS

Using untreated well water in slaughter processes but few treat by using filters and chlorine. All of them have emergency electricity supplier. Most of them have meat inspections by government meat inspectors.

Table 1: Hygienic practice on poultry slaughtering processes.

	Numbers of slaughterhouses [%]
Ante mortem inspection	4/8 [50%]
Stunning process	1/7 [14.3%]
Clean bleeding knife every cut	2/7 [28.6%]
Poultry shall not contact floor during processes	2/7 [28.6%]
Completely defeathered	4/4 [100%]
Completely evisceration	7/8 [87.5%]
Carcass shall not contaminated by offal content	2/4 [50%]
Post mortem inspection	5/9 [55.6%]
Carcass washing after evisceration	7/7 [100%]
Chilling process for storage	4/9 [44.4%]
Chilling process during transportation	2/8 [25%]
Have packaging	7/9 [77.8%]
Routine cleanliness record available	2/8 [25%]

Most of slaughterhouses have resting area for poultry before slaughtering processes begin. The ante mortem are doing at the resting area or at transported vehicles to check poultry health. If there are any sick poultry, all of them will reject back to farm. The death chicken that found will be given to catfish or throw away. From observation, they don't practice on animal welfare because of feather plucking before slaughtering. Most of them slaughter on the floor. The carcasses contact to the floor almost all slaughtering processes. Post mortem inspection do after evisceration but internal organ and carcass doesn't follow together. Chilling process is less practice because of hot meat selling. The chill process is use refrigerator, chilling room and ice. All of slaughterhouses use plastic bag to contain carcasses. Very few of them label

on packages. The cleaning process after operation, only few have routine record. All of them do routine cleaning process by using water and detergent mixed with disinfectant.

Table 2: Hygienic practice on worker personal hygiene.

	Numbers of slaughterhouses [%]
Have short nail	6/8 [75%]
Wear clean and protecting cloth	9/9 [100%]
- Apron	4/9 [44.4%]
- Full hair coverage bouffant cap	2/9 [22.2%]
- Mask	7/9 [77.8%]
- Boots	8/9 [88.9%]
Not smoking in production area	3/9 [33%]
Have general health check	3/9 [33%]

Most of slaughterhouse's worker have short nail and dress clean cloth. From observation, workers usually wear the same cloth from their home without changing it. Apron, bouffant cap and mask are less concern to wear during the slaughtering processes. Boots is the more concerned by worker. Few slaughterhouses have strictly on changing cloth to the slaughterhouses' cloth and wear cap, mask, bouffant cap and boots before slaughtering. Smoking in production area is easy seen in slaughterhouses. Only few slaughterhouses are not allowed to smoke in slaughterhouse's processing area. Most of workers don't have routine general health check. The slaughterhouses have no health check regulation for worker. Very few slaughterhouses have health check regulation. The health check is done by doctor at the hospital and didn't take any samples from the worker. Most of workers are stop working on the sick period at home.

Very few slaughterhouses provide area for sick poultry because of their ante mortem inspection at transported vehicles and rejected sick chicken. Most of them separate dirty zone and clean zone and also separate the entrance. But the separation isn't completely from observation. Most of them provide adequate hand washing facilities such

as sinks for hand wash. The equipment and utensil washing area is less providing. They usually wash at slaughtering area during or after slaughtering processes. Only few slaughterhouses have specific area as washing area. Only one slaughterhouse has non contaminated storage facilities for meat. Most of slaughterhouses use storage containers as plastic bag. The liquid waste management is well concern in all slaughterhouses. They use wastewater treatment system or septic tank with filter before release water to the environment. The water has been check by using catfish as the indicator of water before release in the wastewater treatment system. The solid waste has been given to catfish pond (not into wastewater treatment system), do the fertilizer and given to people who want it.

Table 3: Hygienic practice of slaughterhouse structure.

	Numbers of slaughterhouses [%]
Provide sick poultry area	2/8 [25 %]
Separate dirty zone and clean zone	6/9 [66.7 %]
Separate entrance between dirty zone and clean zone	6/9 [66.7%]
Provide adequate hand washing facilities	6/8 [75 %]
Have equipment and utensil washing area	4/9 [44.4 %]
Have non contaminated storage facilities	1/6 [16.7 %]
Have liquid waste management	9/9 [100%]
Have solid waste management	5/9 [55.6%]

Knowledge on hygienic practices and food safety of slaughterhouse's owner

All slaughterhouses' owner had been interview by using questionnaires and in-depth interview. Most of them accepted that they have been train on hygienic practices and food safety. The training programs that they take are organized by government. Most of owners know meat quality, food safety, proper slaughtering processes and GMP. The major mention is meat quality. The less concerned issues are HACCP and sanitation. Very few of them don't know any hygienic practice and food safety. But they want to have more training on food safety and meat quality. All owners want to improve their worker knowledge. The major training topic that they want worker to been train are hygienic practice and food safety.

Perception on food safety and quality assurance system

Slaughterhouse owners had been interview using questionnaires and in depth interview. Owners are concern on food safety and quality assurance system such as GMP. Most of them think food safety is related to consumers' health, meat quality and effects on finance. The major benefits if they apply food safety and quality assurance system are better product quality and better business profit. But the difficulty to follow food safety and quality assurance system are employee knowledge, equipment, finance, infrastructure, consumer's behavior and waste management. Most of them accept that consumer is the main drive to develop slaughterhouse. Very few want to develop by their own drive. Most of them want the improvement and support to follow the system. The major support that they want is financial support. They also want more support on equipment and training program.

Slaughter poultry market

Slaughter poultry market has only one place from 4 districts because of slaughtering processes was not allowed in 3 districts. From observation, slaughter poultry market is a large poultry market which sells lived birds and providing some area for slaughtering. Consumer buy lived chicken and bring to slaughter area. They have to pay for slaughtering to slaughter man. At slaughter area which is the open room, the slaughter processes are done in the same area without separate dirty and clean zone. Hygienic practices are less concern by workers such as poultry shall not contact to the floor all the processes, non -smoking habit. After slaughter chicken carcass has washing and contain in plastic bag before handing to consumers.

Waste management is not practice at slaughter area.

DISCUSSION

A hygienic practice is the major concern of all slaughterhouses but still have poor practice. Slaughter behavior in most of all is slaughter on the floor. Carcass has been contact to the floor almost of the time. The offal content can contaminate to carcass because of slaughtering processes. The slaughterhouses try to separate dirty zone and clean zone but the incomplete separation still can make cross contamination. The nurhadi's study showed that the average of total plate count among chicken carcasses sold in Yogyakarta market was 1x106 CFU/g which is higher than the standard limitation (7). Workers have less concern on hygienic practice from observation. Smoking habit and no changing clothes are major points that observed. Slaughterhouses should have worker health check regulation. Even they have been train on food safety and proper slaughtering processes; they still less attend to follow. Most of them focus on meat quality and finance. Most of owners still have misunderstanding on food safety and quality assurance system. They have concern on food safety and consumer health but in hygienic practice, they still less practice. They have good perception to follow if they need support to develop slaughterhouses. A good and reliable quality of food chain is much more valuable that they need to follow. Food safety issues should be the direct responsibility of and controlled by the poultry farm and slaughter plant by using HACCP principles (8, 9). The food safety and quality assurance system including Hygienic practice in poultry slaughterhouse need more impetus.

CONCLUSION AND RECOMMENDATION

The situation on poultry slaughterhouses in Yogyakarta is on the improvement on the standard, facilities, equipment and slaughtering behaviors. Food safety issues should be the other main responsibility of the slaughterhouses and increase the important equal to meat quality and using HACCP principles. However, the improvement need to have the regularly data records and regularly recheck on slaughter processes. Knowledge and perception on standards, food safety and quality assurance system are average good and continuously increase. The awareness of community and consumer's demand on meat quality are the great effect to develop slaughterhouses.

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Implementation of an EcoHealth Approach for a Better Management of Leptospirosis in Kulon Progo District, Yogyakarta Special Province, Indonesia

DyahAyu Widiasih[1,*] Wayan T. Artama[2,*] Bambang Sumiarto[1] AdiHeru Husodo[3,*] Pande Made Kutanegara[4,*] GuntariTitik Mulyani[7] Estu Widodo[7] Fihirudin[7] Aris Purwantoro[2,*] Krishna Agung Santosa[5,*] TjutSugandawaty Djohan[6,*] Herman Sancoko[3] Ratna Ermawati[7,*] Paulina Duhita Anindita[2] Fred Unger[8] Jeff Gilbert[8]

[1] Department of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Gadjah Mada
[2] Department of Biochemistry, Faculty of Veterinary Medicine, Universitas Gadjah Mada,
[3] Department of Public Health, Faculty of Medicine, Universitas Gadjah Mada,
[4] Department. of Anthropology, Faculty of Cultural Science, Universitas Gadjah Mada,
[5] Department of Socio-Economic, Faculty of Animal Sciences, Universitas Gadjah Mada,
[6] Department of Ecology, Faculty of Biology, Universitas Gadjah Mada,
[7] Graduated student of Veterinary Science Studies Program, Faculty of Veterinary Medicine, Universitas Gadjah Mada,
[8] International Livestock Research Institute (ILRI)
*Corresponding author: EcoHealth Resource Center-Universitas Gadjah Mada (EHRC-UGM),
Email: dawidiasih@yahoo.com

ABSTRACT Outbreaks of leptospirosis in Yogyakarta Special Province, Indonesia cause serious problems in humans and animals. With case fatality rates for the Kulon Progo District, Yogyakarta Special Province of 5.8% for 2011, 7.1% for 2012 and 33.3% for 2013 the PH importance has been clearly demonstrated. High mortalities in human due to renal failure can be observed whereas in animals a loss of production (i.e. due to abortions) is most prominent. The observed re-emergence of leptospirosis seems to be caused by multifactor components, and highly linked to environmental factors. However, observed cases of leptospirosis and related health impacts need to be better understood. Classical sector specific approaches have failed to be effective. Thus a more successful control and prevention requires alternative concepts such as EcoHealth to ensure transdisciplinary and participatory approaches across sectoral barriers. The objective of this study is to explore potential risk factors for leptospirosis in human and animals by using qualitative and quantitative tools for data collection. In-depth interviews (IDI) targeted on knowledge of zoonotic diseases particularly leptospirosis and behavior of people. Focus group discussions (FGD) were performed and included farmers, village officials, and stakeholders from Municipal Health Services and Livestock Services. The survey was carried out in between April to May 2013 in selected communities (N=9) of Kulon Progo with high (N=3), medium (N=3) and low cases (N=3) of human leptospirosis based on a recent serological survey. It is anticipated that the results from the FGD and IDI will provide additional information to the first phase of the project (Oct 2011 – May 2012) which focused on quantitative data collection such as prevalence surveys for leptospirosis in animals, questionnaires

and spatial analysis using GIS including veterinarians, physicians, ecologists, demographist, social sciences, others stakeholders (government and public sector). Key results from both phases will be presented and synthesized. The synthesis from the qualitative and quantitative surveys will contribute to a better integrated understanding of disease emergence and the basis for a more effective addressing of public health policy concerns in order to better management the disease.

KEYWORDS: *EcoHealth concepts, Focus Group Discussion, Leptospirosis, Kulon Progo District, Yogyakarta*

INTRODUCTION

Recently outbreaks of human leptospirosis in Yogyakarta Special Province during 2011 have been increasing significantly, particularly in Bantul and Kulon Progo District, the southern areas of Yogyakarta Special Province. In Kulon Progo District case fatality rates were 5.8% for 2011, 7.1% for 2012 and 33.3% for 2013. For the data above, it could be considered leptopsirosis have public health concern. Generally, mortality of Leptospirosis in Indonesia reaches 2.5% - 16.45 % or the average of 7.1 %. This number can reach 56 % in cases more than 50 years old. High mortalities in human due to renal failure can be observed whereas in animals a loss of production (i.e. due to abortions) is most prominent. The observed re-emergence of leptospirosis seems to be caused by multifactor components, and highly linked to environmental factors. Leptospirosis is most common in tropical and subtropical areas with high rainfall and humidity. In developing countries, where the health of the environment less attention especially landfills, Leptospira will easily grow and therefore it is often called with the rural disease (1).

The emergence of new infectious diseases such as leptopsirosis, considered largely driven by socio-economic factors, environment and ecology. However, observed cases of leptospirosis and related health impacts need to be better understood. Recently, leptopsirosis has priority to be controlled and it is in the fifth rank by Ministry of Agriculture, Indonesia, meanwhile it is in the third rank by Ministry of Health, Indonesia (before April 2013, new reg. in 12 rank).

Classical sector specific approaches have failed to be effective. Thus a more successful control and prevention requires alternative concepts such as EcoHealth to ensure transdisciplinary and participatory approaches across sectoral barriers. We need a new approach to the such emergence of new infectious diseases; study in this transdisciplines that are framed within biomedical, social, economic, political and cultural contexts is necessary for prevention and control efforts.

The objective of this study is to explore potential risk factors for leptospirosis in human and animals by using qualitative and quantitative tools for data collection.

MATERIALS AND METHODS

This study has been performing participatory of physicians, veterinarians, ecologists, socio- economic experts, demographist, anthropologist, and other stake holders to explore risk factors that influence prevalence of leptospirosis. Methodologies were divided into 2 phases:
First phase (Oct 2011 – May 2012):

Quantitative data collection including surveillance of leptospirosis prevalence in animals, questionnaires were addressed on demographic data of farmers, livestock information, nutrition management, barn management, and history of the disease that led to be potential risks of leptospirosis prevalence and spatial analysis based on positive cases distributed around the river and carried buffer (distance positive leptospirosis cases with watershed, at distance of 50ms, 100ms, 200ms, 300 ms and more than 400 ms,

respectively) using GIS that involved veterinarians, physicians, ecologists, demographist, social sciences, others stakeholders either government and public sector.

Second phase (April to May 2013)

In-depth interviews (IDI) on knowledge of zoonotic diseases, and behavior of people were distributed to the farmers, community and stakeholders to explore risk factors and background of endemicity of prevalence of leptospirosis which targeted on knowledge of zoonotic diseases particularly leptospirosis and behavior of people. Focus group discussions (FGD) were performed and included farmers, village officials, and stakeholders from Municipal Health Services and Livestock Services. The survey was carried out in between April to May 2013 in selected communities (N=9) of Kulon Progo with high (N=3), medium (N=3) and low cases (N=3) of human leptospirosis based on a recent serological survey. Length of IDI average in 1.5 - 2 hours with average 15 people in each groups.

The results from the FGD and IDI will provide additional information to the first phase of the project.

RESULTS AND DISCUSSION

The first phase of the project the result were summarized below:

Surveillance and screening agents from animals

The preliminary results showed that the prevalence cattle sera that postive tested in MAT were 10%, sheep and goat sera were 1.6% (1/60), and dog and cat sera were 0% (0/60), respectively. Several kinds of serovars has been determined that serovarHarjo (52%), Ichterrohaemorrhagiae (18%), Rachmati (16%), Bataviae (5%), Javanica (2%), and Pomona (2%), respectively (Table 1.). The high prevalence of *Leptospira interrogans* serrovar Hardjo were 52% in Kulon Progo District. It could be considered that cattle in Kulon Progo District area were the most carrier animal of *Leptospira* sp., due to the host specific of Leptopsira serovar Harjo mainly in cattle. Leptospira hardjo is one of the biggest causes of leptospirosis in cattle in the United

States (2) and cause infertility cases or abortion in Indonesia, Iran, USA, Canada, Turkey, Brazil and Mexico (3-8).

Table 1: Prevalence of *Leptospira* sp. in animals.

Leptospira serovar	Percent (%)
Hardjo	52
Icterohaemorrhagiae	18
Rachmatie	16
Battaviae	5
Javanicae	3
Pomona	2
Celledoni	2
Tarrasovi	2

Demographic data

The preliminary results from questionnaires from the farmers involved in this study showed that mainly the age of farmers were ≥ 55 years old (65.03%), the most education they engaged were primary school (52.45%) and the occupation mostly were farmers (97.2%) as shown in table 2.

Table 2: Demographic data of farmers involved in the study.

	Percent
Age (years)	
≤ 45	3.50
46-55	31.47
≥ 5	65.03
Education	
Not passed primary school	3.50
Primary school	52.45
Elementary school	36.36
High school & higher	7.69
Occupation	
Farmers	97.20
Traders/ enterpreneurs	2.80

Potential risks from animal

The results of questionnaires observed showed that cattle have potential risk of leptospirosis from their daily habits such as grazing in the field, bath in the river, used for work, feed by grass that grown in the field that have so many mice, and also number of sick cattle that positive tested in MAT (9). The most significant risks are the positive number of cattle that harbored *Leptospira* sp. and significantly proofed by Odds ratio.

Spatial analysis of leptospirosis

The distribution of leptospirosis in Kulon Progo district showed the mapping of the

disease based on the carried buffer examined. Number of leptospirosis cases at a distance of 50 meters was found 12 cases, at a distance of 100 m was found 23 cases, at a distance of 200 m was found 15 cases, and 3 cases were found in distances greater than 300 m. In comparison with the result from the prevalence of *Leptospira sp.* in animals and the secondary data from human cases, it could be concluded that there River Progo that determined by using distance buffer.

The second phase of the project showed the qualitative data on risk factors that influence prevalence of leptospirosis. IDI results were summarized below:

Knowledge of zoonotic diseases and leptospirosis

Some people understand that there are some animals that can cause disease to human, such as rabies which is spread by dogs, avian influenza which is spread by poultry and also they knew "rat disease", a local name for leptospirosis.

Mostly farmers from all the selected areas knew little about leptospirosis. They knew it caused by rats, but they did not know other animal and livestocks even human could spread the disease through their infected urine. They also noticed that some people from neighborhood have been infected by leptospirosis, among those were died.

The knowledge on leptospirosis transmission is also varies mostly spread by rats urine, rice field water, contact with infected animal, but still confusing whether all species of rats could also spread it. Rats mostly found in the roof of the house, backyard, moor pile of grain warehouse and also in the rice field. Species of rats found varies from *Rattus argentiventer* (local name = wirog), *Rattus rattus* = pithi, and *Rattus norvegicus* (= clurut) and *Rattus tiomanicus* (mostly in the tree) mostly pithi were found at home. They are all local rats. Since the prior knowledge of farmers on leptospirosis were varied, it could be one of the risk factors of leptospirosis spreading in that area.

Behaviour of people

Mostly farmers in the low and medium human cases area have unhealthy habbits, do not have habbit of washing hands after works, or contact with animals, in contrast with the people in high human cases area. Waste carelessly discarded outside the home, collected in the holes within 10 m from the house before being burned.

Control and prevention

Several attempts have been carried out to eradicate rat population such as using natural predators (snake, garangan/cerpelai (Ind) = *Herpestes javanicus* or owl). This way faced some obstacles that animal predators often sought by human for sale, either for food or as pets. Other ways for rats eradicating are killing, burning and being burned by traditional fireworks. There is still some problem faced, when rats were killed by poison at the rice field, then the other rat colonies will revenge by ruining the vehicles electricity cables.

Stakeholder policy/program

Both authorized personnel's from Municipal Health Services and Livestock Services have been held socialization or extension as effort for control measure of the outbreaks of leptospirosis. They emphasized community education of zoonotic diseases.

The results from the FGD and IDI will provide additional information to the first phase of the project.

CONCLUSION

Key results from both phases will be presented and synthesized. The synthesis from the qualitative and quantitative surveys will contribute to a better integrated understanding of disease emergence and the basis for a more effective addressing of public health policy concerns in order to better management the disease. Therefore the implementation of Ecohealth approach is considered to be the best tool for better management of emerging infectious disease.

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Ecosystem Approaches to the Better Management of Brucellosis and Toxoplasmosis in Yunnan Province, China

Wengui Li[1] Unger Fred[2] Xiangdong Yang[3] Shibiao Yang[4] Jeffrey Gilbert[2] Jing Fang[5] Guorong Yang[6,*]

[1] College of Animal Science and Technology, Yunnan Agricultural University, Kunming, China
[2] International Livestock Research Institute, 30709, Nairobi, Kenya
[3] Yunnan Institute of Endemic Disease Control and Prevention, Dali, China
[4] Yunnan Animal Science and Veterinary Institute, Kunming, China
[5] The Institute for Health and Development Sciences, Kunming Medical University, Kunming, China
[6] Yunnan Academy of Grassland and Animal Science, Kunming, China
*Corresponding author; Email: ynygr@126.com

ABSTRACT Brucellosis and Toxoplasmosis are zoonoses of concern in many countries of Asia and Southeast Asia including China despite of ongoing control efforts. To face this challenge, new approaches are required such as Ecohealth targeting on transdiciplinary collaboration. In the presented research, a part of ILRI's Ecozd project, veterinary, public health, animal science experts from five provincial institutions, practitioners from the project sites, and policy authorities from the provincial Department of Health and Department of Agricultural worked together to understand perception, awareness and behavior of stakeholders, investigate the impact of these diseases and demonstrate how an integrated approach can help to improve collaboration between different stakeholders and contribute to a better control. The research was carried out in Mangshi County and Yiliang City of Yunnan and research activities consisted of historical data review, biological sampling, questionnaires, Focus Group Discussion and In Depth Interview. Also Outcome Mapping was used as an evaluation tool for changing of behavior of targeted groups. Results from biological sampling indicate Brucellosis as a PH hazard anyhow sporadic, while Toxoplasmosis is endemic, both in ruminants and human in the selected regions. Potential risk factors for the spread of these zoonoses exist. Awareness and zoonoses knowledge of potential risk groups was in general low. The use of a "learning by doing" EcoHealth approach in this study has improved team members capacity on Ecohealth and its practical realization in a field study. Outcome mapping indicated a change of behavior in targeted groups, especially on zoonoses knowledge and willingness to share of information.

KEYWORDS: *Ecohealth, Toxoplasmosis, Brucellosis, Transdiciplinary*

INTRODUCTION

Southeast Asia is considered a hotspot for emerging zoonotic diseases, rapid economic and population growth creates conditions and drivers for disease emergence (1). Like other parts of China, top-down administrative approaches which usually focus on sector or discipline are responsible for the prevention and control of zoonoses. Animal and human health sectors are operating through their

own independent systems. Despite of ongoing control efforts, Brucellosis and Toxoplasmosis are still concerned zoonoses in many parts of China, including Yunnan. For instance, human Brucellosis occurred mainly around the border with Inner Mongolia, but endemic regions gradually spread to some southern provinces and more non-epidemic provinces have become epidemic during 2005-2010(2).As classical control efforts have failed to face this challenge, alternative approaches are needed such as Ecohealth (EH) targeting on transdiciplinary collaboration. Here we present experiences in implementation of a study using an Ecohealth approach(3) with the objective to investigate the importance of Brucellosis and Toxoplasmosis in pilot communities and hospitals, to understand perception, awareness and behavior of stakeholders including potential risk groups towards these zoonosis, to demonstrate how an integrated approach can help to improve collaboration between different stakeholders, and thus consequently contribute to the building of a strong EcoHealth network for prevention and control of zoonosis in China.

MATERIALS AND METHODS

Performance of this presented study started in July 2010 and will end in August 2013. Based on a review of available secondary data and expert opinion related to Brucellosis and Toxoplasmosis, Yiliang and Mangshi County were selected as study areas(Fig. 1). Yiliang County is 55 kilometers away from Kunming, with an elevation of 1540 meters and a north subtropical monsoon climate, 90.9% of the population is ethnic Han and dairy ruminants are mainly cow and goats. Comparatively, Mangshi City is 679 kilometers away from Kunming, bordering Myanmar, 51.06% of populations are ethnic groups (e.g. Dai). Elevation is approximately 900meters and a subtropical monsoon climate zone, diary ruminants are mainly water buffalo.

Following an EH approach, a framework was developed in a joint effort anda set of different quantitative and qualitative survey
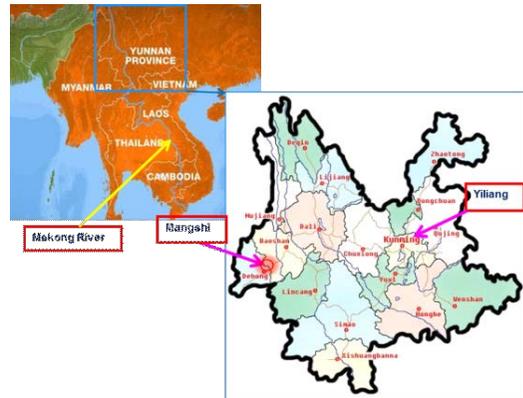
Figure 1: Location of Yiliang county and Mangshi City in Yunnan province of China.

tools were used apart from this biological sampling. Within each county 2 township and within township at least 2 villages were selected based on animal population data. Management data were collected from dairy farmers by semi structures questionnaires from both study areas (N=192). Focus Group Discussion (FGD, for villagers with and without animals) and In-depth Interview (IDI, for village vets and human doctors) gained information on perception and awareness in risk groups towards zoonoses and cross-sectional collaboration. Biological samples included bulk milk from selected dairy farms (buffalo, goat and cattle). Human serum was collected from volunteers involved farmers, workers, village vets or doctors to get first-hand information on Brucellosis and Toxoplasmosis in those communities. In addition, information of suspected clinical cases and historical prevalence data for both zoonoses were collected from hospital (N=4) and vet stations (N=16) located in both counties (Fig. 2). Outcome Mapping(4)was used as an evaluation tool for changing of behavior of targeted groups.

RESULTS

Historical data review indicated more than 35,000 human Brucellosis cases been reported in China in 2010. Although there are only few reported cases for animals and human for Brucellosis in Yunnan the trend is increasing year by year. Historical data for Toxoplasmosis

Figure 2: Groups involved and Information collected.
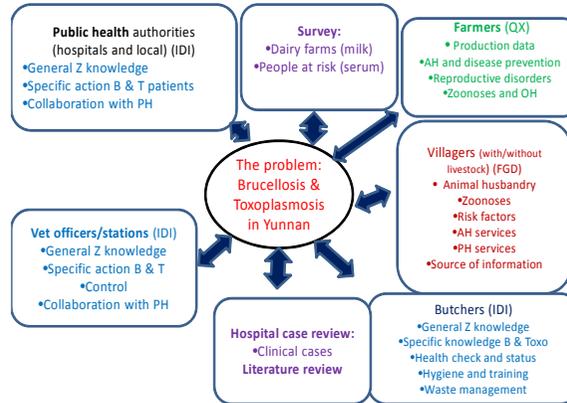
in Yunnan indicate 26.4-46.4% in swine, 1.4-23.3% in beef cattle, and 8.7% in occupation and 3.6% in general human population (5,6).

Serological epidemiology survey results indicated that in Yiliang County, antibody prevalence rate of Brucellosis and Toxoplasmosis were 3.97% (5/126) and 1.59% (2/126) in human, 2.4% (1/41) and 34.1% (14/41) in dairy goat, 11.4% (5/44) and 18.2% (8/44) in dairy cow, respectively. A human Brucellosis case linked to owned cows was include in antibody positive samples of farmers, this case has been reported by County CDC timely. In Mangshi City, there are 0% (0/102) and 5.88% (6/102) in human, 0% (0/41) and 7.3% (3/41) in diary buffalo, respectively.

FGD and Questionnaire survey found that work experience of farmers is short as 1-3 years, or higher as over 20-30 years. In Yiliang County, the villagers are averagely in a higher level of education, and extensive experience in feeding and management of domestic animal. While Mangshi is located in border of southwest China, the level of economic development is relatively low. Reasons for farmers to raise ruminants include incentives by local government, and higher earnings from ruminants compared to other domestic animals like pigs or poultry. Risk factors for the spread of zoonosis exist. For instance, toilets of Dai nationality in Mangshi are usually built on rivers, excreta are directly released into drainage system. Other traditions include consumption of raw meat is popular in certain

communities and few people were found like to drink raw milk. There was a general low awareness of zoonosis, only very few farmers know Brucellosis and Toxoplasmosis. Limited knowledge on surveillance, quarantine and milk/meat borne disease. Veterinary services are readily accessible, farmers satisfied with the service.

In depth interview of village vets& human doctors and butchers revealed also a general very low awareness and limited knowledge of zoonotic diseases. The most frequently mentioned zoonotic diseases are Rabies, Avian influenza. But also Foot and mouth disease was stated. PH and vet officials indicated that there is a governmental monitoring and reporting system for infectious disease. However, most of village doctors and vets are not aware of the existence of this system, which may be caused by the no-participatory nature of this system. There is lack of diagnostic criteria and laboratory test for Brucellosis and Toxoplasmosis and other zoonotic diseases at village and township levels of both veterinary and health systems. Little communication and collaboration between both departments are prominent. There is only little training on zoonotic diseases, in particular related toBrucellosis and Toxoplasmosis. Outcome mapping results revealed that team members have an increased understanding of EcoHealth principles due to the implementation of the project, character as actively engage in policy-making process locally and nationally, seek

opportunities to disseminate research findings and communicate about EcoHealth research process to other institutions.

Results from outcome mapping also demonstrate that village doctors and veterinaries realize the importance of strengthen the cooperation between both departments, also the need to improve diagnostic and treatment for zoonoses.

Village heads are willing to assist organize of meetings and trainings. Suspected cases of diseases and other abnormal condition are reported timely. Village head may also have a leading role in changing from unhealthy and unhygienic behavior and assist in the construction of sanitary pens and standardized dairy buffalo raising area. They can also help farmers to apply for budget support from local government to build sanitary public toilet.

DISCUSSION

Constant presence of emerging or reemerging zoonosis in SE Asia proved that if human can't pay sufficient attention to the state of ecosystems and the social and economic inequities between people who depend on these same ecosystems, efforts to improve global health and human development will falter (1). To face this challenge, new approaches are required such as Ecohealth which offer the opportunity to break the exiting disciplinary silos that are often observed in the research and development communities including China. Using of Ecohealth approach will bring stakeholders work together and increase the transfer of research results into zoonotic disease management.

Performance of this EcoZD projects faces many challenges. The limited Eco-Health capacity of partners from participating institutions was a key challenge. Besides, complex collaborative partnerships between academic, college, government, community and other stakeholders are another challenge. To overcome these challenges, crucial was the support of Provincial Agriculture Department and Health Department. The enforced continued communication by the coordinating institute throughout the entire project was another condition for success. Those challenges could also overcome through multiple leaders and champions who collaborate on key tasks, ensuring that each separate unit builds commitment and buy-in to the overall transdisciplinary mission (7).

As the outlined process to achieve a clear picture of two target diseases status, results of literature review, retrospective investigation of human health in hospital and animal health in veterinary station in agreement with bio-sampling, study results indicate that Brucellosis is lower prevalence and morbidity while Toxoplasmosis is higher prevalence and morbidity of in both animal and human. It is necessary for managers to concern the spread of Brucella and Toxoplasma from ruminant to human through milk.

Alarming situation found in this project like most interviewees appeared to be lack of knowledge on zoonosis, especially Brucellosis and Toxoplasmosis. Indicate that disseminate the knowledge of zoonosis, idea of Ecohealth and building up collaboration between stakeholders are in urgent need in Yunnan Province of China.

CONCLUSION

1)   Information for two target zoonoses and their impact on pilot communities and hospitals has been collected. Brucellosis is still sporadic has been clearly demonstrate as a PH hazard, while Toxoplasmosis is endemic, both in ruminants and human in the selected regions

2)   Enrolled stakeholders and risk groups had low awareness and limited knowledge of zoonotic diseases. Limited knowledge on surveillance, quarantine and milk/meat borne disease found in farmers.

3)   Team members' capacity on Ecohealth and its practical realization in a field study has been improved greatly. This found practical evidence in building up of collaboration between involved institutions and stakeholders.

4)   Outcome mapping indicated a change of behavior in targeted groups,

especially on zoonosis knowledge and willingness to share of information.

5)   This study can act as a model for an integrative approach to better control of zoonosis. It is anticipated that this project will guide other partners of China to further recognize the Eco-Health concept and how to implement it.

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Field Building Leadership Initiative: Advancing Ecohealth in Southeast Asia - the First Year Journey

Hung Nguyen-Viet[1,2,*] Giang Huong Pham[1,3] Tung Xuan Dinh[4]

[1] Center for Public Health and Ecosystem Research (CENPHER), Hanoi School of Public Health (HSPH), Vietnam
[2] Swiss Tropical and Public Health (Swiss TPH), Basel, Switzerland; International Livestock Research Institute (ILRI), Nairobi, Kenya
[3] Vietnam Public Health Association (VPHA), Vietnam
[4] National Institute of Animal Science (NIAS), Vietnam
*Corresponding author: Hung Nguyen-Viet, email: hung.nguyen@unibas.ch

ABSTRACT   The Ecosystem approach to health, or Ecohealth is a useful tool to address health and environmental issues in developing countries, particularly in South East Asia (SEA), where agricultural intensification processes have profound implications for ecosystems and health. The Field Building Leadership Initiative (FBLI) Advancing Ecohealth in SEA program aims to build the field of Ecohealth by integrating research, training, policy and networking to focus on solving human health problems associated with agricultural intensification in SEA countries. This paper aims at updating the one-year journey of the program from October 2011 until October 2012 and providing insights into the plan for the coming years. A number of activities of the project's research component, capacity building, and knowledge translation have been completed in line with the proposal. Outcomes and outputs of the one-year implementation are also presented.

KEYWORDS: *Ecohealth, FBLI, interdisciplinary, human health, agricultural intensification*

INTRODUCTION

The Ecosystem approach to health promoted by the International Development Research Center (IDRC) has only recently been introduced in South East Asia. Ecohealth is the transdisciplinary and systematic study of dynamic relationships and interactions among animals, ecosystems and human health. It provides both a theoretical framework for understanding the changing "landscape of health" and a practical approach for identifying solutions to manage interlinked ecosystem and health problems. Charron named six principles of an Ecosystem approach to health: system thinking, transdisciplinary research, participation, sustainability and gender and social equity (1). This approach is a useful tool to address health and environmental issues in developing countries, in particular in SEA where agricultural intensification processes have profound implications for ecosystems and health. However, using the Ecohealth approach to comprehensively tackle complex issues requires individual, institutional, and country capacity in understanding and applying this approach within the context of the region (2).

As a hotspot for (re)emerging infectious diseases (EIDs), the SEA region has hosted a number of Ecohealth projects such as APEIR (Asian Partnership on Emerging Infectious Diseases Research), EcoZD (Ecosystem Approaches to the Better Management of Zoonotic Emerging Infectious Diseases in SEA), EcoEID (Ecohealth emerging infectious diseases research in SEA), and BECA (Building Capacity in Ecohealth for SEA). This

development in the region shows the dynamic landscape of research and application of Ecohealth in various fields such as emerging and zoonotic diseases, agriculture and health, education and training. However, no Ecohealth project has yet integrated a solid link among research, training, policy and ecohealth leadership. The Field Building Leadership Initiative in South East Asia (*FBLI in SEA*) is a new 5-year (2012 to 2017) project funded by the IDRC. Its objectives are to conduct transdisciplinary Ecohealth research, strengthen Ecohealth training, influence policy related Ecohealth issues, and share knowledge among institutions and organizations at national and regional levels. This paper aims to introduce the FBLI and report its one-year journey, as well as discuss some lessons learned from the activities implemented.

MATERIALS AND METHODS

A literature review was performed. The literature used consisted of academic papers, books on the Ecohealth approach and the FBLI first year project report. Rather, in-person or Skype meetings between members of the projects provided opportunity to gain information. The FBLI first year report served as the most important tool for data and information gathering. The objective of this paper is to provide an update on the past year of the project; the target audience includes the community of Ecohealth practitioners, researchers, local stakeholders, and partners of the FBLI family. This work limits itself to discussing the one-year time frame of the project from October 2011 to October 2012.

Background -Field Building Leadership Initiative in South East Asia

The FBLI in SEA is a research project, co-implemented by seven institutions/universities in Thailand, Indonesia, Vietnam and China. The initiative uses a diverse range of methods and activities, including the "site-based research" concept, which is supported by national and regional networking events. The FBLI implements three interlinked components: (i) research – serving as the backbone of the initiative for other

components and employing ecohealth approaches to address ecosystem and health issues related to agricultural intensification, (ii) capacity building - strengthening Ecohealth capacity and leadership through university degree training, training of trainers (ToT) activities, and future leaders training and development, and (iii) knowledge translation - promoting dialogue between the research and policy communities, and building capacity to translate research evidence to inform and influence policy decisions. Figure 1.A captures the relationship among the components.
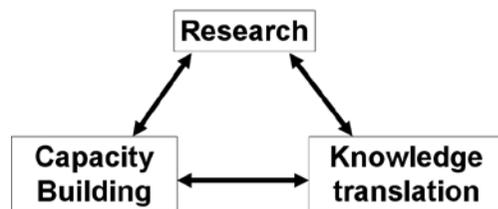
Figure 1.A: Inter-linkage among three components.
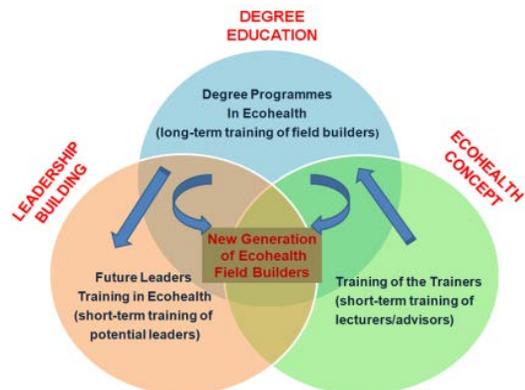
Figure 1.B: The capacity building component.

A Regional Core Group (RCG) – comprised of representatives from a consortium of seven partner institutions from the region with experience in Ecohealth implements the program in close collaboration with national partners, other regional and global Ecohealth networks, and the IDRC.

The FBLI anticipates outcomes and impacts in five key areas, namely, development of a sustainable dynamic Ecohealth field, implementation of research findings and

practical solutions, provision of a robust critical mass of training materials, and establishment of strong linkages among policy makers and researchers in the field. It features four interlinked components that correspond to our program objectives. The operational structure of the program is broad, encompassing regional and local level involvement. The RCG partnership includes seven institutions: the Health System Research Institute (HSRI), the Ministry of Public Health, Thailand, the Center for Public Health and Ecosystem Research (CENPHER), Hanoi School of Public Health (HSPH), the Center for Excellence for Vectors and Vector-Borne Diseases, the Faculty of Science, Mahidol University, the Institute for Health and Development Studies, Kunming Medical University, China, the Kunming Institute of Botany, China, the Faculty of Public Health, Universitas Indonesia, and Veterinarians without Borders, Canada. In the project, the RCG and the Coordinating Unit (CU) are located at the CENPHER, HSPH and closely intertwined. The former consists of representatives from the seven aforementioned institutions collaborating in information and research data exchange as well as development of the regional network, while the latter is set up to support the consistent collaboration and overarching goals of the whole project.

RESULTS

First year implementation activities (October 2011 to October 2012)

Research team building and research topic re-definition

The highlight of results from the research component was the identification of research issues by all research teams during the research inception workshop in Kunming in October 2012, which provided an opportunity for all teams to share their personal experiences and revisit their research plans, leading to the reshaping of research questions and topics. The research topics presented by the four countries included: (i) China: Using ecosystem approach to reduce pesticide use and its health and environment impacts in

Yuanmou County, Yunnan Province, (ii) Indonesia: Ecohealth and Diary Production: Connecting Issues and Finding Interventions for Small-scale Farming in a Southeast Asian Context, (iii) Thailand: Study on potential impacts and proposed best practices in agricultural development: A case study of rubber plantation expansion and increased risk of vector-borne disease in eastern Thailand, and (iv) Vietnam: Using Ecohealth Approach for Better Management of Livestock and Human Waste in Ha Nam province.

In these four countries, research plans were discussed among research teams and other stakeholders through formal and informal meetings. Challenges arose among team members due to differences in backgrounds, and study sites and topics went through multiple revisions and required several field meetings, reflecting one of the biggest challenges in Ecohealth research.

Box 1: *Changing the study site of the Vietnam research team*

"Using Ecohealth Approach for Better Management of Livestock and Human Waste in Ha Nam province, Vietnam" is the research project of the team at the Center for Public Health and Ecosystem Research (CENPHER), Hanoi School of Public Health (HSPH). Ha Nam province has been, for a duration of time, a research site for the team as it also served as the study site for a previous project, on human waste and sanitation conducted by the National Centres of Competency in Research (NCCR) North-South (3), (4).

Initially, at the time of finalizing the FBLI proposal, the Vietnam team had chosen another location, Chi Linh district, in Hai Duong province – the location of the field laboratory of the HSPH. However, after a number of field investigations, the team decided to change the location to Ha Nam province because the results of the previously conducted NCCR project could support their work with FBLI. Additionally, due to the similarity of the two research projects, it was determined that the projects could complement each other. The team could also benefit from the relationship with the local stakeholders.

Capacity building through training

The capacity building component is centered around development of the Ecohealth Trainer Manual and efforts to mainstream Ecohealth as a module or short course at the institutional level. To prepare for the debut of the Ecohealth Trainer Manual, a number of workshops were carried out with the participation of members from different country teams. From October 2011 to July 2012, lecturers/trainers attended various workshops in Thailand, including the *Writeshop* in Bangkok and the *ToT Workshop* in Pattaya, to work on content development for the training manual. This component is also marked by the various courses/modules on Ecohealth delivered under the degree training and future leadership sub-component.

At Mahidol University, Thailand and Universitas Indonesia, the Ecohealth approach is either integrated into other courses as a module or taught as a separate course. Concurrent with the launching of Ecohealth courses/modules was the initiation of the operation of the website and e-platform for sharing materials on Ecohealth. The website (www.ecohealthnetwork.org) is administrated by Mahidol University. 'Future leader' was the training module developed and led by the Universitas Indonesia. Some literature about leadership was reviewed and upon this review, the leadership module draft was developed and presented at the RCG meeting in Thailand. In the development of this module, which is distributed in both English and Bahasa Indonesian, the Faculty of Public Health, Universitas Indonesia, collaborated with the Lembaga Psikologi Terapan (LPT UI).

Box 2: *The FBLI Ecohealth Trainer Manual and Regional Ecohealth/One Health in SEA*

The manual was developed by a group of researchers working in the field of Ecohealth, including FBLI team members, and led by Veterinarians without Borders/Vétérinaires Sans Frontières (VWB/VSF), one of the seven partner institutions in the project. The manual is 'part of a global initiative to build capacity in ecosystem approaches to health' and has a 'focus on how to teach Ecohealth, providing teachers and trainers with starting point from

which to explore, improvise, adapt, and develop diverse educational Ecohealth teaching experiences for and with their participants'(5).

The manual has been reviewed by different users to ensure that it adequately addresses user needs.

Cooperation with APEIR and Knowledge translation

The APEIR Coordinating Office was in charge of the Knowledge Translation component of the project and, is credited with having synthesized the first five APEIR research projects, which will include a policy brief for each project and results' dissemination through a series of workshops at national and regional levels. The research findings from the FBLI will be synthesized and disseminated by APEIR in collaboration with other partner institutions in the project.

DISCUSSION

Participatory and transdisciplinary approach in the FBLI-initial lessons learn

From the outset, the project has stated that 'field building can be viewed as the ongoing process of empowering individuals, institutions, and countries with stakeholders who have diverse knowledge systems.' A combination of short-term and long-term strategies – directed at different actors and levels – is required for delivering a sustainable field of Ecohealth research in the region. In this on-going process, a participatory process which 'helps identify barriers to change, clarifies information and knowledge gaps'[i]is necessary. The result of the participation process in this case is the behavior change of the researchers, which is reflected in the re-visitation of their research topic and methodologies. It is said in the first technical report "the research teams will have to conduct a deeper exploration of the issues at the community level by re-visiting the study sites and spending more time with the community to identify their needs for research".

It should be noted that the FBLI proposal to the IDRC used a number of Outcome

Mapping (OM) related concepts like boundary partners, progress markers, and the identification of 'what you expect to see, what you would like to see, or what you would love to see' as the outcomes(6). Key boundary partners were identified as young researchers, students, university lectures and leaders, mid-career professionals, senior researchers, local line agencies, communities, mid-level decision makers and research funders. These boundary partners were identified as those with whom 'the initiative interacts and influences directly'. It was concluded that changes were seen at the researcher level at the research inception workshop.

Other kinds of participatory approach in the project can be seen through the series of workshops that were organized to achieve the launching the Ecohealth Trainer Manual. In two FBLI workshops, the *Writeshop - Review of Ecohealth Trainer Manual* (29-31 March 2012, Bangkok, Thailand), and the *TOT Ecohealth Workshop for lectures/trainers* (30 June to 4 July 2012, Pattaya, Thailand), organized by the VWB/VSF, it is interesting to note that the aims were to encourage the participation of different types of stakeholders. The former aimed at a review of the draft manual from the perspective of potential users (future trainers), students and others involved in the field of Ecohealth research and education, and the latter 'provided a forum for future Ecohealth trainers/lectures to collaborate as teams to prepare to teach Ecohealth courses'. Here we can see that different stakeholder participation add to the knowledge generated by the research and enhanced the action that can result from, or be integrated into, the research.

CONCLUSION AND WAYS FORWARD

The FBLI project has completed a quarter of its route map. There have been achievements as well as challenges. Year 1 has reflected some characteristics of the practice of the ecosystem approach to health, e.g. the implementation of the interdisciplinary as well as the participatory approaches. As noted through the various workshops, participants valued the collaborative and interactive format as a way of learning from each other and building relationships; this should be maintained in coming up workshops. At times, the diversity in the team members' background became a challenge. For some teams, this was the first time applying a real Ecohealth approach. Many of the teams realized the difficulty of applying the transdisciplinary approach, as it required not only negotiations to reach a consensus among scientists, but also the engagement of non-scientists (community and local leaders). Therefore, the strong leadership and coordination of the country project team leaders and commitments of team members are an essential factor for timely project implementation.

In this first year period, some of the unexpected outcomes included the collaboration among the FBLI partner institutions and others. Additionally, the Ecohealth approach has gained attentions from researchers working in other fields such as the One-Health group.

In the future, the teaching of Ecohealth in academic institutions will be fostered. The curriculum on Ecohealth is expected to be officially processed through Mahidol University and will be officially opened to the public by 2013. Both coursework and non-coursework research plans will be included with priority given to the former. The translation and distribution of the Ecohealth Trainer Manual into different local language has also been prioritized. This should help widen the distribution of the issue to the local Ecohealth community. In the next three years, it is expected that the project will result in more outputs and outcomes in all components. It is also suggested that monitoring and evaluation activities should be carried out in order to track and report the outputs and unexpected outcomes of the project periodically.

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Health Information Communication in Northern Thai Highland Communities: A Longitudinal Socio-economic Perspective

G. Lamar Robert[1*]   Chongchit Sripun Robert[1*]

[1] *EcoHealth-One Health Resource Centre, Faculty of Veterinary Medicine, Chiang Mai University*
Corresponding author; Email: lamar.chiangmai@gmail.com; chongchit.robert@gmail.com

ABSTRACT   Health information communication within and among northern Thai hilltribe communities in Chiang Mai, Chiang Rai, and Mae Hongson provinces has gone through a major transition over the past half century. This longitudinal synthesis paper, based on information obtained between 1980 and 2013, explores exogenous and endogenous socio-economic factors which affected health information communication in highland communities. Data was collected using qualitative approaches including direct observation, in-depth interviews, and focus groups plus quantitative surveys conducted with members of hilltribe communities, Thai government officials, and development workers in the highlands. Initially, geographic isolation, language issues, and lack of government health services meant that most health information communication occurred within individual villages.   Then beginning in the 1960s, opium crop replacement projects brought expanded infrastructure (roads, electricity, radio and television, schools, and health facilities) which increased communication with the outside world. Organizations such as Heifer International Thailand distributed both livestock and training on animal health and zoonotic disease prevention. Networking promoted by the Micro Economic Development Foundation provided another channel for inter-village health information communication. In the 1990s, cellphones and the internet further facilitated communication, including contact with lowland health care providers. Some members of hilltribe communities, both men and women upon completing their studies at high schools and universities returned to their villages, communicating information about of modern medicine. Present health information communication challenges include the growing sanitation risks from increasingly crowded villages, zoonotic risks from free roaming pigs, etc., and documenting traditional hilltribe health care information to make it available to future generations.

KEYWORDS: *communication, health information, hilltribes, socio-economics, networking*

INTRODUCTION

Several distinct ethnic groups, each with their own language and culture, live in the highland areas of northern Thailand. A series of socio-economic transitions over the past half century have had significant impact on communication related to human and animal health both within and among hilltribe

communities as well as between hilltribe communities and the ethnic Thai majority in the lowlands. This study aims to provide a longitudinal description of hilltribe health information communication.

MATERIALS AND METHODS

This study represents a synthesis of the research and development activities in hilltribe communities in Chiang Mai, Chiang Rai, and Mae Hongson Provinces. Data was collected using qualitative approaches included direct observation, in-depth interviews, focus groups, plus quantitative surveys conducted with members of hilltribe communities, Thai government officials, and development workers in the highlands. The data collection was accomplished as an adjunct to studies of different hilltribe development efforts over the period 1981 to 2013. Informants have included members of hilltribe communities, Thai government officials working in highland areas, and staff members of highland development projects.

RESULTS

Prior to about 1960, communication between minority hilltribe communities and the ethnic Thai majority living in the lowland areas of the country was minimal. Although hilltribe communities were usually quite tight-knit because their survival depended on cooperation, individual villages were often widely separated geographically. What communication occurred among villages was chiefly among members of the same ethnic group, in part because each group has its own distinct language. Government policy toward the hilltribe population at that time could perhaps best be described as benign neglect: virtually nothing was required of the hilltribes, e.g., they paid no taxes, they were not conscripted to serve in the Thai military, etc. Similarly, little or no government services such as health care and education were provided. Villages grew crops for home consumption, mainly rice, corn (as feed for pigs), and vegetables. They raised some livestock, primarily pigs and chickens, allowing the animals to roam freely. Their diet was supplemented by edible plants and hunting wildlife from surrounding forest areas. Communication of health information was largely limited to that provided by oral traditions and the wisdom of the village traditional healer with a strong reliance on herbal remedies.

Even when epidemics occurred, there was usually no communication with government health agencies. One example is the case reported by the Skaw Karen of Ban Huai Tong, Mae Win Sub-district, Mae Wang District in Chiang Mai province. They explained that in 1957, after several people in the village had died of unknown causes, a number of households relocated to their present location in Ban Huai Tong. Their reaction to an apparent disease epidemic is very similar to the response to bubonic plague in late middle ages (1340-1400) (1). Response to the death of a woman in a Skaw Karen community has similar health overtones. Traditionally, among that ethnic group when a woman dies in a house, that house must be destroyed. The intent to stop the spread of disease is implicit in this custom, but no effort is made to communicate with health officials to determine the actual cause of death.

Transport of a sick or injured individual from a highland village to a lowland health facility was infrequent due to the distances involved, problems in communicating in the Thai language, and a lack of funds to pay for health care. As trips to lowland towns were undertaken only once or twice a year to obtain basic commodities such as metal cooking pots, cooking oil, and salt, most villages were unaware of government provisions for waiving health care fees for those unable to pay.

Beginning in the 1960s, in reaction to increases in consumption of heroin[ii] in western countries, opium crop replacement projects were initiated in cooperation with the Thai government to eradicate opium production in highland areas.[iii] Most of the development programs included a health communication component. For example, the Thai-German Highland Development Project (TG-HDP) and Heifer International promoted

stall-raising of pigs as a zoonosis prevention measure (2), (3). The traditional system of allowing pigs to roam free and find their own food created a risk of parasites in the feces of the pigs being transferred to villagers, who often walked barefoot. Stall raising reduced health risks, but increased costs in terms of labor: villagers had to obtain and prepare food for pigs, e.g., chopping up and boiling banana trees. In addition, they had to deal with the disposal of pig waste. In some communities where communication of the benefits of stall-raising of pigs has not yet been effective, pigs can still be seen running freely through the village.[iv] The Micro Economic Development Foundation (MEDF), as part of a program to establish village level savings groups, also promoted networking among highland villages. In addition to sharing knowledge related to savings and micro loans, the networks established by MEDF also provided another channel for inter-village health information communication.

Some of the changes occurring concurrently with the opium crop replacement project had a negative impact on hilltribe health. Traditionally, most hilltribe groups practiced some form of shifting cultivation, clearing an area of forest to crops for a few years.[v] The field would then be allowed to fallow for years or even decades before again being cleared for agriculture. Even entire villages relocated periodically, insuring not only soil fertility but also a good supply of edible and useful forest products. It was during this time that the Thai government began to strictly enforce regulations prohibiting clearing forest areas for agriculture and relocating villages. The enforcement of those regulations made it necessary for farmers to learn an entirely new system of agricultural production. Without the application of fertilizer, soil fertility on hillside fields rapidly declined, reducing crop yields. As villages could not be moved, resources near the village, including edible plants and animals, soon became over-harvested. The combined result was a sharp decline in overall health: a 1987 TG-HDP health and nutrition study found a high incidence of nutrition-

related problems such as angular stomatitis, chronic runny noses, and chronic under-nutrition of young people, problems not fully addressed by indigenous knowledge(4).

The Thai government during this period did begin significant efforts to understand the health problems in highland areas and to communicate health information to hilltribe communities. One good example is the government response to a widespread problem of goiter caused by iodine deficiency. Goiter resulting from iodine deficiency was identified as a serious problem in TG-HDP project villages in Mae Hongson province. In one Shan village, almost 60% of the individuals examined had goiter (5). The government response was to institute a program to produce and promote the use of iodized salt. That program was widely communicated in hilltribe villages, with iodized salt soon becoming available in small village shops.[vi]

Health information communication was further augmented by new health centers established by the Thai government in many highland areas. These centers were staffed by health professionals trained in modern medicine, increasing the flow of health communication to hilltribe communities. However, the health center officials, with very few exceptions, had little knowledge of the traditional health practices of the communities they served.[vii] That resulted in an unforeseen impact on health information: a reduction in interest in and communication of traditional health knowledge. For example, the ethno-botanist Dr. Edward Anderson during his fieldwork for his book, *Plants and People of the Golden Triangle*, interviewed traditional healers in hilltribe villages in northern Thailand (6). He noted that although the traditional healers' knowledge of traditional health practices was extensive, not one of them had a younger member of the community learning that knowledge as an apprentice.[viii]

Newly established government schools provided another channel for the communication of health information. The schools taught "health information" lessons

on such topics as the need to drink clean water. Unfortunately, as the school curricula established by the Ministry of Education was uniform throughout the country, and included no mention of traditional hilltribe health care practices. Even had it been possible to include traditional health, most teachers in highland schools were not highly motivated to learn about or teach that information: young graduates of teachers colleges frequently applied for highland school positions as a means of becoming a government civil servant. Then, as soon as they were able, most applied to transfer to a lowland school.

Roads were another factor affecting health communication in the highlands.

Traditionally, villagers walked along forest trails to get to lowland towns. Then, with the advent of development projects, dirt access tracks were opened leading to hilltribe villages. Those dirt roads were suitable only for four wheel drive vehicles; in the rainy season, chains and a winch were also required to access many villages. As few hilltribe households could afford such vehicles, the new roads did not initially increase the number of highland people traveling to lowland hospitals for health care. Later, as a still on-going program of paving highland roads began, access to lowland towns became easier. The all-weather roads allowed highland cash crops such as cabbage, baby carrots, and coffee to be easily transported to lowland markets. Marketing of these crops not only improved the economic situation of hilltribes, it also greatly improved hilltribe community access to and communication with lowland hospitals and other health facilities.

By the 1990s, there were many advances in communication both among highland villages and between highland and lowland groups. Health centers and even hospitals were constructed in or near highland communities, greatly expanding access to modern medicine. In addition, government policy was modified to allow schools to include local content in curricula, including segments on traditional health knowledge.

The Thai government in the mid 1990s developed plans to extend telephone landlines to rural areas, including hilltribe communities (7). Those plans were soon abandoned with the advent of the cellular phone system. Today, cellphone coverage is nearly universal in the highlands, significantly facilitating the exchange of information about health matters between hilltribe communities and medical professionals. There are only a few areas where it is necessary to travel a short distance to an area where a cellphone signal is available. Electricity has been extended to all but the most remote villages and most highland schools now have computers and internet access proving another channel for health information communication.

In the past decade, many members of hilltribe communities, both men and women, have gone to lowland areas to continue their education. Some individuals, upon completing their studies at high schools and universities, have returned to their villages to live, bringing with them knowledge of modern medicine which they communicate to others in the community. During recent (May 2013) focus group interviews with hilltribe groups in Chiang Mai, Chiang Rai, and Mae Hongson provinces no evidence of an education-based schism was observed. The knowledge of those with a high school or college education was valued by individuals with little or no education. Similarly, the traditional wisdom and knowledge of those with little formal education was appreciated by those who had received advanced schooling. That situation suggested the existence of a robust two-way communication of health information.

In spite of all the changes in health information communication, there are still challenges remaining. During field visits to hilltribe villages in 2012 and 2013, pigs were still seen roaming freely in some communities indicating that additional information on zoonotic risks is needed. Additionally, it was seen that many hilltribe communities are becoming increasingly crowded as it is no longer possible to clear additional forest areas. That increased population density, combined with the very basic sanitation facilities in most communities, represents a risk for the spread

of infectious disease. Finally, because of increases in communication of modern medicine, knowledge of traditional health practices, particularly the use of herbal remedies, is declining. No efforts were observed in any communities to document traditional healing practices.

DISCUSSION

Health information communication in northern Thai highland communities has been affected by both endogenous and exogenous factors. Endogenous factors impeding health information communication among ethnic hilltribe groups included differences in culture and language, e.g., sharing tribe-specific information on herbal remedies. The geographic separation of the communities further limited communication. Those same factors, culture, language, and distance, similarly created barriers to health information communication between hilltribe communities and the ethnic Thai majority in lowland areas.

Exogenous factors have also impacted communication of health information. Changes in agricultural production systems, particularly the change from shifting cultivation to permanent agriculture, affected the health of the communities. Opium crop replacement projects together with the extension of government services to highland areas have introduced all-weather roads, schools, health centers, electricity, radio and television, telephones, and networks of savings groups helped expand opportunities for communication of traditional and modern health information both among hilltribe villages and between those communities and lowland areas.

CONCLUSION

Overall the social and economic changes over the past fifty years which have affected health information communication among hilltribe communities in northern Thailand and between those communities and lowland areas appear, in the long run, to have been beneficial to their health and well-being. However, in spite of the increases in health information communication, important challenges remain. One challenge is a holdover from traditional livestock raising practices: allowing pigs to roam freely through villages. The zoonotic risks of pigs wandering freely have not been sufficiently embedded in the consciousness of some communities. A second challenge that increased population density in some hilltribe communities, with their very basic sanitation facilities, presents a risk for the spread of infectious diseases. The final challenge is that the increased communication of modern medicine concepts has reduced interest in traditional health information. That indigenous knowledge, e.g., medicinal plants, traditional remedies, etc., if not documented soon, may be unavailable for communication to future generations.

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ii Ten kilograms of opium can be refined into one kilogram of heroin.

iii Traditionally, opium, which grows well in Thailand at elevations at or above 1,000 meters,had been produced primarily for local consumption. Villagers were paid only small amounts for the opium they produced, with the real profits going to the middlemen who went to the villages to purchase raw opium. Countries conducting development programs included the U.S., Germany, Norway, and Australia, as well as international organizations such as the United Nations

iv During a May 2013 project evaluation trip to several hilltribe villages in Chiang Mai, Chiang Rai, and Mae Hongson provinces the researchers observed pigs running free in several villages.

v The exception is the Karen whose villages are located at lower altitudes where they practice irrigated rice production and remained in one location. Other ethnic groups practice rainfed agriculture on sloping fields.

vi Each packet was marked "Iodized Salt" and bore the Caduceus symbol of the Thai Ministry of Health and contained iodine at a ratio of 1:50,000. There was an attempt to take advantage of the situation. In some hilltribe village shops for a short period salt packets with a bogus seal very similar to that of the Health Ministry, but indicating an iodine content of only 1:1,000,000, one fiftieth of the recommended concentration, were offered for sale.

vii In the one case the health center official was himself a member of a hilltribe community, the individual was a Hmong but he was serving a community of ethnic Lahu and Lisu. Knowledge of traditional medicine is very much specific to each ethnic group. In one field trip with Dr. Anderson, shamans from two different ethnic groups separately identified medicinal plants in the same approximately 100 meter radius area. Each shaman identified about two dozen plants; virtually none of the plants and their uses overlapped. The publication that resulted from this fieldwork is *Plants and People of the Golden Triangle* (Anderson, 1993).

viii An indication of the diversity of indigenous knowledge of the traditional healers is that on one field trip shamans from two different ethnic groups separately identified medicinal plants in the same approximately 100 meter radius area. Each shaman identified about two dozen plants, but virtually none of the plants or their uses overlapped.

POSTER PRESENTATIONS
Food Safety and Food Security

Prevalence and Virulence-associated Gene Profiling of *Streptococcus suis* in Pigs Slaughtered for Consumption in Chiang Mai and Lamphun, Thailand

Wichanee Chanto[1,*] Duangporn Pichpol[2,3] Kanruethai Wongsawan[4] Reinhard Fries[5]

[1] *Joint Master Course in Veterinary Public Health (MVPH) of Freie Universität Berlin and Chiang Mai University, Thailand*

[2] *Division of Veterinary Public Health, Department of Veterinary Biosciences and Veterinary Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand*

[3] *Veterinary Public Health Centre for Asia Pacific (VPHCAP), Faculty of Veterinary Medicine, Chiang Mai University, Thailand*

[4] *Division of Veterinary Paraclinic, Department of Veterinary Biosciences and Veterinary Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand*

[5] *Institute of Meat Hygiene and Technology,* Faculty of Veterinary Medicine, *Freie Universität Berlin, Germany*
Corresponding author; Email: wichanee.c@gmail.com

ABSTRACT

Streptococcus suis is now regarded as a zoonotic pathogen of concern in Thailand and elsewhere. The objectives of this study were to determine the prevalence of *S. suis* isolated from pigs slaughtered for consumption and genetic characteristics of this agent based on the presence of 3 virulence-associated genes; extracellular protein factor (*epf*), Muramidase-released protein (*mrp*), and Suilysin (*sly*). From a total of 220 tonsils from pigs slaughtered at 12 slaughterhouses in Chiang Mai and Lamphun during December 2012 to April 2013. *S. suis* was initially identified by microbiological techniques. Multiplex PCR targeting the gene encoding glutamate dehydrogenase (*gdh*) and serotype-specific genes of serotype 1 or 14, 2 or 1/2, 7, 9 16 was performed for confirmation and serotyping. The 3 virulence-associated genes were also investigated by Multiplex PCR. *S. suis* was isolated from 38.2% (84/220) of the tonsils collected. Serotype 16 (2.7%) was the most prevalent serotype followed by serotype 7 (2.3%), serotype 2 or 1/2 (1.4%) and serotype 9 (0.9%) respectively. Based on the presence of 3 virulence-associated genes, 7 various genotypes were found. Most of *S. suis* strains harboured the *epf-mrp- sly* - genotypes. This study demonstrated *S. suis* as a genetically diverse agent. Even though *S. suis* serotype 2 or 1/2 which is the most virulent strain causing human infections was found in only 1.4%, slaughter pigs are still considered an important key in the epidemiology of the infection due to its carrier capacity of various virulence-associated genes of *S. suis*. Awareness regarding *S. suis* infection needs to be extended to the public.

KEYWORDS: Carrier, Pigs, Prevalence, Streptococcus suis

Prevalence and Antimicrobial Susceptibility of *Listeria monocytogenes* in Fresh Poultry Products in Bandung, Indonesia

Yoni Darmawan Sugiri[1,*] Josef Kleer[2] Greta Gölz[2] Tongkorn Meeyam[3,4] Warangkhana Chaisowwong[3,4] Thomas Alter[2]

[1] *Joint Master Course in Veterinary Public Health, Freie Universität Berlin and Chiang Mai University, Thailand, and Balai Pengujian dan Penyidikan Penyakit Hewan dan Kesmavet (BP3HK), Dinas Peternakan Provinsi Jawa Barat, West Java, Indonesia*
[2] *Institute of Food Hygiene, Department of Veterinary Medicine, Freie Universität Berlin, Germany*
[3] *Department of Veterinary Biosciences and Veterinary Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Thailand*
[4] *Veterinary Public Health Centre for Asia Pacific (VPHCAP), Faculty of Veterinary Medicine, Chiang Mai University, Thailand*
Corresponding author; Emails: yoni_dvm@yahoo.com, yonidvm@gmail.com

ABSTRACT

The purpose of this study was to determine the prevalence and the number of *Listeria monocytogenes* in 184 samples of fresh poultry products sold in traditional markets and supermarkets in Bandung, West Java, Indonesia, and also to determine the antimicrobial resistance pattern and molecular characterization of the isolated *L. monocytogenes* strains. The samples were analyzed following ISO 11290-1: 1996 and ISO 11290-2: 1998. Disc diffusion method was applied for antimicrobial susceptibility test and multiplex polymerase chain reaction was applied for molecular serotyping of the isolated *L. monocytogenes*. Overall *L. monocytogenes* was detected in 15.8 % of the fresh poultry products, with prevalence of 15.2 % and 16.3 % for traditional markets and supermarkets samples respectively. There was no significant difference between traditional markets and supermarkets regarding the prevalence and the count number of *L. monocytogenes*. The average *L. monocytogenes* count in poultry products sold in traditional markets was 1.08 log cfu/g, and 1.03 log cfu/g for supermarkets. The contamination level of *L. monocytogenes* was < 10 cfu/g in 87.5 %, between 10 and 100 cfu/g in 9.8 %, between 100 and 1,000 cfu/g in 2.2 %, and > 1000 cfu/g in 0.5 % of the samples. All 29 isolates in this study belong to the molecular serogroup IIb comprising the serovars 1/2b, 3b and 7. 27.6 % (8/29) isolates were resistant to at least one of ten antimicrobials tested, and 72.4 % (21/29) of the isolates were susceptible to all antimicrobials tested. Among 29 isolates, 17.2 %, 6.9 %, 6.9 % and 3.4 % were resistant to penicillin, ampicillin, erythromycin, and a combination of ampicillin and penicillin respectively. The results of this study reveal that there is wide spread cross contamination and emerging antibiotics resistant of *L. monocytogenes* isolated from fresh poultry products sold in study area. There are strong needs to improve this condition by improving good hygienic practices and prudent use of antimicrobial drugs at all level of poultry production chain.

KEYWORDS: *Listeria monocytogenes, Poultry products, Antimicrobial susceptibility, Molecular serotyping, Multiplex PCR*

Quantifying *Salmonella* Contamination in Pig Slaughterhouses in Hung Yen, Vietnam

Sinh Dang Xuan[1,2,*] Hung Nguyen-Viet[2] Tongkorn Meeyam[3,4] Reinhard Fries[5]

[1] Joint Master Course in Veterinary Public Health (MVPH) of Freie Universität Berlin, Germany and Chiang Mai University, Thailand

[2] Center for Public Health and Ecosystem Research (CENPHER), Hanoi School of Public Health, 138 Giang Vo, Hanoi, Vietnam

[3] Department of Veterinary Biosciences and Veterinary Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Thailand

[4] Veterinary Public Health Centre for Asia Pacific (VPHCAP), Faculty of Veterinary Medicine, Chiang Mai University, Thailand

[5] Institute of Meat Hygiene and Technology, Freie Universität Berlin, Brümmer Str. 10, Berlin D-14195, Germany

*Corresponding author; Email: dxs@hsph.edu.vn, xuansinhck@gmail.com

ABSTRACT

The risk of *Salmonella* contamination along the pork production is a worldwide concern. Not only the biological contamination, but also personal perception and individual behavior play a role for risk assessment along a chain. This study is to identify the prevalence and number of *Salmonella* in 3 pig slaughterhouses in relation to several risk factors. During January to April 2013, a total of 87 samples (carcasses, workers' hands, cutting board and belly skin material) were collected from 4 visits to each slaughterhouse. A 3-tube Most Probable Number (MPN) was applied to quantify the number of *Salmonella* from carcass, workers' hands, cutting board and belly skin samples (35 samples). The result showed that *Salmonella* prevalence was 36.9% (26.7-47.8%), mostly found on worker's hands (50%) and the lowest from cutting board (33.3%). The highest number of *Salmonella* on carcass and cutting boards was less than 0.075 MPN/cm^2 and 1.2 MPN/cm^2, respectively. Potential risk factors were tested however they were not statistical significant. This study underlines the necessity of good hygiene practices and management in slaughterhouses. In terms of food safety, further risk assessment of *Salmonella* in pork production chains is needed.

KEYWORDS: *Hygienic practice, pig slaughterhouse, risk factors, Salmonella, Vietnam*

Prevalence and Antimicrobial Resistance of *Salmonella* spp. in Slaughtered Pig in Pork Production in Chiang Mai and Lamphun, Thailand

Min Thit Lwin[1,*] Peter Paulsen[2] Duangporn Pichpol[3,4]

[1] *Joint Master Course in Veterinary Public Health (MVPH) of Freie Universität Berlin and Chiang Mai University, Thailand*

[2] *Institute for Meat Hygiene, Meat Technology and Food Science, Department of Farm Animals and Public Health in Veterinary Medicine, University of Veterinary Medicine Vienna*

[3] *Veterinary Public Health Centre for Asia Pacific (VPHCAP), Faculty of Veterinary Medicine, Chiang Mai University, Thailand*

[4]*Department of Veterinary Biosciences and Veterinary Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Thailand*

*Corresponding author; Email: dr.minthitlwin@gmail.com

ABSTRACT

A cross-sectional study was conducted to determine the proportion of antimicrobial resistance of *Salmonella* spp. of slaughtered pig in pork production in Chiang Mai and Lamphun provinces, Thailand during December 2012 to April 2013. A total of 111 caecal content samples were collected from 111 pig butchers for different flock of pigs, from 12 registered slaughter houses of Department of Livestock Development, Thailand. Caecal content samples were taken immediately during evisceration. *Salmonella* spp. was identified by the method slightly modified to ISO 6579:2002. Ten different antimicrobial agents belonging to 6 pharmacological groups including penicillin, tetracycline, cephalosporin, aminoglycoside, sulphonamide and quinolone were used for antimicrobial susceptibility test. Antimicrobial susceptibility test was carried out by the disk diffusion method of CLSI, 2012. *Salmonella* spp. was recovered from86.5% (96 in 111). The most isolated *Salmonella* resistant to Ampicillin (84.4%, 94 in 111). No isolated salmonella resistant to norfloxacin (0%, 0 in 111). Moreover, this study found 92.7% (178 in 192) and 44 patterns of multiple antimicrobial resistances. Two isolated *Salmonella* were resistant to 8 antimicrobial agents and 6 isolates were susceptible to all 6 group antimicrobial agents. The study indicated that multiple antimicrobials resistant *Salmonella* spp. should be concern and publish to consumers, farmers and slaughterhouse's owners who get involved in pork production in Chiang Mai and Lamphun provinces.

KEYWORDS: *Antimicrobial susceptibility, Pig feces, Slaughter houses, Salmonella* spp.

Prevalence and Antimicrobial Resistance of *Vibrio* spp. in Retail Shrimps in Hanoi, Vietnam

Tra Vu Thi Thu[1,*] Duangporn Pichpol[2,3] Ngan Pham Hong[4] Stephan Huehn[5] Thomas Alter[5]

[1] Joint Master Course in Veterinary Public Health (MVPH) of Freie Universität Berlin and Chiang Mai University, Thailand

[2] Veterinary Public Health Centre for Asia Pacific (VPHCAP), Faculty of Veterinary Medicine, Chiang Mai University, Thailand

[3] Department of Veterinary Biosciences and Veterinary Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Thailand

[4] Department of Veterinary Public Health, Faculty of Veterinary Medicine, Hanoi University of Agriculture, Vietnam

[5] Institute of Food Hygiene, Faculty of Veterinary Medicine, Freie Universität Berlin, Germany

*Corresponding author; Email: thutra_ty48c@yahoo.com.vn

ABSTRACT

Vibrio spp. are natural inhabitants of the aquatic environment. Among the members of the genus, twelve species have been reported to be pathogenic to humans and can cause foodborne infections. The aim of the recent study was to investigate the prevalence and the antimicrobial resistance patterns of *Vibrio* spp. isolated from retail shrimps in Hanoi, Vietnam. A total of 202 shrimp samples were collected from retail markets located in ten urban districts of Hanoi. Among those, 201 (99.5%) samples were positive for *Vibrio* spp. The most common species detected was *V. parahaemolyticus* (95.5%), followed by *V. alginolyticus*(56.4%), *V. cholerae* (2%) and *V. vulnificus* (1.5%). Multiple *Vibrio* spp. were found in 114 (56.4%) samples. In total, 195 *V. parahaemolyticus* isolates, four *V. cholerae* isolates and three *V. vulnificus* isolates, which are *Vibrio* species associated with foodborne infections, were tested for resistance to eight antimicrobial drugs using the disc diffusion method. *V. parahaemolyticus* isolates showed a high rate of resistance against ampicillin (87.2%), while a moderate rate was observed for sulfamethoxazole/trimethoprim (18.5%) and intermediate resistance towards tetracycline (24.6%). Low resistance rates (0.5%) were recorded against both ciprofloxacin and cephalothin. Only one *V. cholerae*isolate with resistance to ampicillin and two *V. cholerae* isolates to sulfamethoxazole/trimethoprim were found. All of the *V. vulnificus* isolates (n=3) were susceptible to the eight antimicrobial agents tested. Among the three *Vibrio* species, multi-resistance was found only in *V. parahaemolyticus* (16.9%). The result of this study indicates the high prevalence of *Vibrio* spp. in shrimp resistant to likewise ampicillin and sulfamethoxazole/trimethoprim.

KEYWORDS: *Vibrio, prevalence, antimicrobial resistance*

First Reported Prevalence and Antimicrobial Resistance of *Campylobacter* spp. in Fresh Chicken Meat in Nueva Ecija, Philippines

Fredelon Bunnao Sison[1,2,*] Warangkhana Chaisowwong[3,4] Suruda Tiwananthagor[3,4] Duangporn Pichpol[3,4] Kannika Na Lampang[3,4] Greta Gölz[5] Thomas Alter[5]

[1] *Joint Master Course in Veterinary Public Health (MVPHCAP) of Freie Universitaet Berlin and Chiang Mai University, Thailand*

[2] *College of Veterinary Science and Medicine, Central Luzon State University, Nueva Ecija, Philippines*

[3] *Department of Veterinary Biosciences and Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand*

[4] *Veterinary Public Health Centre for Asia Pacific (VPHCAP), Faculty of Veterinary Medicine, Chiang Mai University*

[5] *Institute of Food Hygiene, Freie Universitaet, Berlin, 14163 Berlin, Germany*

*Corresponding author; Email: fredbsison@yahoo.com

ABSTRACT

This study was conducted to determine the prevalence and to semi-quantify *Campylobacter* spp. on chicken breast skin samples at four selected local wet markets and to determine antimicrobial resistance patterns of the *Campylobacter* isolates. Fifty seven out of the 120 samples tested were confirmed to be positive for *Campylobacter* spp. The estimated prevalence was 47.5 (95 % CI: 38.66-56.72). Out of the 57 samples tested, 54.39% (n=31) were identified to be *C. coli* and 45.61% (n=26) *C. jejun*i. Among the four local wet markets, highest prevalence was determined in Cabanatuan with 35.09 %. Almost 53% of the samples tested positive for *Campylobacter* spp. had a contamination of MPN = ∞ (LCL 580/g) (1). With regards to degree of resistance to five antibiotics, out of the 44 isolates tested, 77.27% were resistant to ampicillin which is being the highest, followed by ciprofloxacin 70.45%, tetracycline 54.55%, erythromycin 20.25% and gentamicin 11.36%, respectively. Moreover, 81.82% (n=36) of the isolates were resistant to at least one antimicrobial agent. For two antimicrobial drugs, there were 13.64% (n=6), 38.64% (n=17) for three drugs, 13.64% for four drugs and 6.89% (n=3) were resistant to all five antimicrobial drugs. A similar trend of increasing pattern of multi-resistance was observed in the country and other countries where use of the antimicrobial drugs was moderately unrestricted in both humans and animals. Such high prevalence of *Campylobacter* spp. contamination in chicken meat at retail suggests the need of sanitary handling of poultry meat. Based on these data, we strongly suggest good and efficient intervention measures at slaughterhouses to minimize fecal contamination of broiler skin and decrease cross-contamination.

KEYWORDS: *Campylobacter spp., prevalence, antimicrobial resistance*

Presence of Class I Integrons associated with Norfloxacin- and Ofloxacin-Resistant *Salmonella* from Slaughtered Pig in Chiang Mai and Lamphun, Thailand

Phyoe Thu Aung[1,2,*] Duangporn Pichpol[3] Nattawooti Sthitmatee[3] Tongkorn Meeyam[3]

[1] *Master Student, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand*

[2] *Researcher, Livestock Breeding and Veterinary Department, Ministry of Livestock Breeding and Fisheries, Mandalay, Myanmar*

[3] *Department of Veterinary Biosciences and Veterinary Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand*

Corresponding author; E-mail: phyoehtun@gmail.com

ABSTRACT

Salmonellosis is one of the common bacterial foodborne diseases and plays an important role on economic and public health worldwide. The objective of this study was to identify class 1 integrons and the association with norfloxacin- and ofloxacin resistance of *Salmonella* isolated from pig slaughter house in Chiang Mai and Lamphun, Thailand. A total of 208 *Salmonella* isolates were included in this study. Norfloxacin and ofloxacin resistance were identified using standard disk diffusion method, while class 1 integrons were detected by PCR. The results showed almost 0.40% (1/208) *Salmonella* isolates were resistant to norfloxacin, whereas 8.17% (17/208) was resistant to ofloxacin. Of these isolates, class 1 integrons was detected 37.01% (77/208). The presence of class 1 integrons is significantly not associated with the resistance of norfloxacin (*p*=0.5524) and significantly associated with the resistance of ofloxacin (*p*<0.05). This study suggest that ofloxacin-resistant *Salmonella* was more widely distributed than norfloxacin resistant *Salmonella* and ofloxacin-resistant *Salmonella* was mostly associated with class I integrons which could be contribute to antibiotic resistance among swine population in Chiang Mai and Lamphun provinces.

KEYWORDS: *Antibiotic resistance, Class I integrons, PCR, Salmonella*

Microbiological Evaluation of Hygienic Practices of Pig Slaughterhouses in the National Capital Region, Philippines

Samuel Joseph Manglapus Castro[1*] Tongkorn Meeyam[2,3] Reinhard Fries[4]

[1] *Joint Master Course in Veterinary Public Health (MVPH) of Freie Universität Berlin and Chiang Mai University, Thailand*

[2] *Department of Veterinary Biosciences and Public Health, Faculty of Veterinary Medicine, Chiang Mai University*

[3] *Veterinary Public Health Centre for Asia Pacific (VPHCAP), Faculty of Veterinary Medicine, Chiang Mai University*

[4] *Institute of Meat Hygiene and Technology, Faculty of Veterinary Medicine, Freie Universität Berlin*

*Corresponding author; Email: samcastro_dvm@yahoo.com.ph

ABSTRACT

The continuing growth of the Philippine swine industry and intensification of swine production warrants the maintenance of effective and sustainable measures in slaughter facilities to ensure quality of products and prevention of disease spread. The purpose of this study was to evaluate and compare the hygienic status of two classifications of slaughterhouses, accredited and unaccredited, in the National Capital Region (NCR), Philippines through determination of hygiene indicator microorganisms. Environmental and carcass samples were collected and processed to obtain the Total Viable Count (TVC), enumeration of *Enterobacteriaceae* and detection of *E. coli* and coliforms for water samples, all in accordance to ISO standards. Both facilities were visited five times each from the period of January to April 2013 where a total of 190 samples (40 carcass swabs, 140 environmental swabs, 10 water samples) were collected and processed. The average TVC for all samples was 4.58±1.25 log cfu/cm^2 whereas the overall value of *Enterobacteriaceae* was 2.08±1.44 log cfu/cm^2 (n=180). The mean value obtained for TVC in pig carcasses (4.06±0.95 log cfu/cm^2) was within the range set in guidelines being enforced in the Philippines. Likewise, the mean *Enterobacteriaceae*count on carcasses was 2.32±1.48 log cfu/cm^2, within the acceptable range of those guidelines. Regardless of observable contrasts in terms of structure, equipment and facilities in both types of facilities, results from the study showed TVC and number of *Enterobacteriaceae* count in unaccredited and accredited facilities to be not statistically different in general. The largest number of coliforms (>2300 MPN/ml) and *E. coli* (2300 MPN/ml) were detected in a water sample from the unaccredited slaughterhouse while contamination in the accredited facility was found to be at lower levels. This study seeks to contribute to better understanding of the current hygienic conditions in such facilities, which would contribute to identification of gaps in the system and determine appropriate interventions for improvement.

KEYWORDS: *Hygiene, Slaughterhouses, Enterobacteriaceae, Philippines*

First Results From a Microbiological Assessment of Commercial Poultry Feeds Distributed in Nepal

Anand Kumar Singh[1,*] Mukul Upadhaya[2] Kannika Na Lampang[3] Warangkhana Chaisowwong[3,4] Hafez Mohamed Hafez[5]

[1] Joint Master Course in Veterinary Public Health of Freie Universitoet, Berlin and Chiang Mai University, Thailand
[2] Department of Veterinary Public Health, Ministry of Agriculture, Nepal
[3] Departments of Veterinary Biosciences, Faculty of Veterinary Medicine, Chiang Mai University, Thailand
[4] Veterinary Public Health Centre for Asia Pacific (VPHCAP), Faculty of Veterinary Medicine, Chiang Mai University
[5] Institute of Poultry Diseases, Department of Veterinary Medicine, Freie Universität Berlin, Germany
*Corresponding author; Email: anandsingh85@daad-alumni.de,anandsingh85@yahoo.com

ABSTRACT

Feed production is a major component of the poultry industry because almost about 80% of total cost of production goes to feed but less attention has been paid to the role of bacterial contamination of feed with regard to human food borne illness. This study aimed to identify the level of microbiological and fungal contamination of commercial poultry feeds distributed in Nepal. The study included all registered pellet feed mills located throughout the country. Overall 130 pellet feed and 50 mash feed samples were collected and analyzed. The laboratory methods for microbiological and fungal analyses were performed on the basis of ISO Standards 4833:2003 (APC), ISO 21528-2:2004 (*Enterobacteriaceae*), ISO 7954:1987(yeast and moulds) and ISO 6579:2002 (*Salmonella*). The feed samples were examined for total aerobic plate count, total *Enterobacteriaceae* count, yeast and mold count and detection of *Salmonella* spp. The microbiological analysis showed an average total aerobic plate count of 5.19 ± 0.81 log cfu/g of feed followed by total *Enterobacteriaceae* counts of 3.82 ± 0.78 log cfu/g and total yeast and mould counts of 3.89 ± 0.63 log cfu/ g. Incomparing the level of contamination between 13 feed companies, no statistically significant differences found for APC but there were significant differences observed between feed companies with regards to *Enterobacteriaceae* counts (p = 0.0183) and yeast and moulds counts (p = 1.04×10^{-8}). From this study we also ascertained that the investigation of *Salmonella* spp. in pellet feed did not obtain any positives whereas in mash feed 38% of the samples were positive for *Salmonella* spp. In conclusion, the feed were found to be contaminated and could pose a potential health risk to the poultry and humans.

KEYWORDS: *APC, Enterbacteriaceae, Yeast and Mould, Salmonella, Feed, Nepal*

First Findings on the Prevalence of Extended-Spectrum β-Lactamases Producing *Escherichia coli* (ESBL-producing *E. coli*) and Risk Factors in Dairy Farms in Beijing Area, China

Farong Xu[1,2,*] Uwe Rösler[3,*] Anika Friese[3] Maximilian Baumann[3] Jingyi Zhao[2]Haitao Wei[2] Xiaodong Liu[2] Khwanchai Kreausukon[4,5,*]

[1]*Joint Master Course in Veterinary Public Health (MVPH) of Freie Universitaet Berlin and Chiang Mai University, Thailand*

[2]*Beijing General Station of Animal Husbandry and Veterinary Service, Beijing, China*

[3]*Faculty of Veterinary Medicine, Freie Universitaet Berlin, Berlin, Germany*

[4] *Department of Food Animal Clinic, Faculty of Veterinary Medicine, Chiang Mai University*

[5] *Veterinary Public Health Centre for Asia Pacific (VPHCAP), Faculty of Veterinary Medicine, Chiang Mai University*

*Corresponding author; Email: xufarong@hotmail.com, uwe.roesler@fu-berlin.de, kkhwanchai@yahoo.com

ABSTRACT

For the purpose of investigating the herd prevalence of ESBL-producing *E. coli* in dairy farms in Beijing area, China, a cross sectional study was conducted to detect the frequency of ESBL-producing *E. coli* in fecal samples of dairy farms Beijing area as well as to assess veterinary drug use on these farms. Fecal samples were collected and questionnaires were administered on 99 farms. Feces was diluted by Maximum Recovery Diluent (MRD) and streaked on MacConkey containing 2 mg/L cefotaxime (MAC-CTX) plates to isolate ESBL-producing *E. coli*. Isolates were confirmed by using API 20 E biochemical kits and disk diffusion tests were applied to screen and confirm for ESBLs. Data analysis was performed by using R statistic software. The results showed that in sixteen out of 99 farms (16.2%) ESBL-producing *E. coli* were found. Antimicrobial susceptibility testing was performed and among the 16 isolates, 100% were resistant to cefotaxime and ampicillin, 75% were resistant to streptomycin, 81.3% to kanamycin, 37.5% to chloramphenicol, 56.3% to gentamicin and ciprofloxacin, 50 % showed a resistance to trimethoprim, 43.8% to tetracycline, 62.5% to trimethoprim and sulfamethoxazole combination, 68.8% to sulfamethoxazole, 31.3% were resistant to nalidixic acid, and 87.5 % were multidrug resistant. The study identified that using antimicrobial contaminated milk to feed calves was associated with the detection of ESBL-producing *E. coli*.

KEYWORDS: *ESBL-producing E. coli, Prevalence, Drug resistance, Dairy farm*

Antimicrobial Resistance of *Escherichia coli* Isolated from Broiler at Rajshahi Region, Bangladesh

Muha. AjijurRahman Al Azad[1,*] Ruhul Amin[2] Mst. IsmatAra begum[3] Maximilian Baumann[4]
Kannika Na Lampang[5,6] Hafez Mohamed Hafez[7]

[1] *Joint Master Course in Veterinary Public Health; (MVPH) of Freie Universitaet Berlin, Germany and Chiang Mai University, Thailand*

[2] *Scientific Officer, Bangladesh Council for Scientific and Industrial Research, Rajshahi; Bangladesh*

[3] *Associate Professor. Department of Animal Husbandry and Veterinary Science; Rajshahi University, Bangladesh*

[4] *Faculty of Veterinary Medicine, Freie Universitaet Berlin, Germany*

[5] *Department of Veterinary Public Health and Bio Science, Faculty of Veterinary Medicine, Chiang Mai University, Thailand*

[6] *Veterinary Public Health Centre for Asia Pacific (VPHCAP), Faculty of Veterinary Medicine, Chiang Mai University*

[7] *Institute of Poultry Diseases, Faculty of Veterinary Medicine. Freie Universitaet; Berlin, Germany*

Corresponding author; Email: razad80@gmail.com; r_azad80@yahoo.com

ABSTRACT

Antimicrobial resistance, a major, health problem for both humans and animals throughout the world, is leading to treatment failure after administering antimicrobial drugs. This study was conducted from December 2012 to May 2013 to determine the prevalence of antimicrobial resistance of *Escherichia coli* in live broilers in Rajshahi district of Bangladesh. Five Upazilla were selected randomly and 200 cloacal swab samples of live birds were collected from 50 different broiler farms (four samples from each farm). A flock was classified as resistant to antimicrobials if one of the four samples showed resistance to any of the nine antimicrobials tested for. Isolation of *E. coli* was done by conventional microbiological methods followed by biochemical identification. A total of 200 *E. coli* isolates were collected and tested for resistance to nine antimicrobial agents (ampicillin, erythromycin, tetracycline, gentamicin, ciprofloxacin, levofloxacin, trimethoprim-sulfamethoxazole, colistinsulphate, and streptomycin). Antimicrobial resistance tests were performed by following the standard disc diffusion technique mentioned by the Clinical and Laboratory Standards Institute (CLSI-2011). Results showed that all isolates are multi-drug resistant (≥5 antimicrobial agents) and all were 100% resistant to tetracycline, erythromycin, streptomycin, ampicillin, trimethoprim sulphamethoxazole and ciprofloxacin. The highest sensitivity pattern of 73.5% of the isolates was determined for colistinsulphate followed by gentamycin (49%), and levofloxacin (17%).Results suggest that a high resistance of *E. coli* to antimicrobials exerts a threat to the poultry industry at Rajshahi area in Bangladesh, so raising awareness about proper administration of antimicrobials in broiler farms is crucial from an animal production and veterinary public health point of view.

KEYWORDS: *E. coli, Antimicrobials, Resistance, Broiler, Bangladesh*

Serodiversity of *Salmonella* on Farms, in an Abattoir and Pork in Northern Thailand

Arsooth Sanguankiat[1,4] Samart Dorn-in[2] Wasan Chantong[2] Renu Pingtong[3] Pawin Padungtod[2]
Lertrak Srikitjakarn[2] Reinhard Fries[1]

[1] *Department of Veterinary Medicine, Freie Universitaet Berlin, Germany*
[2] *Faculty of Veterinary Medicine, Chiang Mai University, Thailand*
[3] *Faculty of Agro-Industry, Chiang Mai University, Thailand*
[4] *Faculty of Veterinary Medicine, Kasetsart University, Thailand*

ABSTRACT

Foods of animal origin are commonly produced and consumed in Thailand. Additionally, exporting of swine products is gradually increasing every year since 2003. Safety of pig chain from food borne pathogen such as *Salmonella* is still a concern. This longitudinal study was conducted from December 2004 –May 2005 in Thailand. Twenty two cohorts consisting of 194 finishers were selected conveniently from a large commercial swine production chain. Each cohort consisted of 7-10 pigs, representing a particular herd. Samples were collected on farms (feces, water, wastewater and pen swabs), in an abattoir (carcass swabs after splitting and cleaning, caecal contents, mesenteric lymph nodes, freshly cut meat) and at retail (transported and retail pork). Additionally, environments of a deboning unit in the abattoir were sampled in parallel. Examination for *Salmonella* was conducted following ISO 6579 (2002) with slight modifications. Serological testing was done by slide agglutination. From a total of 971 positive samples, 1000 isolates were available for serotyping. *Salmonella* Rissen was the predominant serovar (45.9%) and found in every cohort. The highest number 44 (4.4%) of *S.* Rissen was found in cohort 8 and 19, whereas the lowest number 4 (0.4%) occurred in cohort 16. Considering this study, elimination of *Salmonella* on farm might reduce the contamination of *Salmonella* down streams, i.e. at slaughter and markets. Improving hygienic practice on farm and in slaughterhouse is crucial to reduce the risk of *Salmonella* in pork chain.

KEYWORDS: *Serodiversity, Salmonella, Farm, Pork, Abattoir*

Occurrence of *Salmonella* spp. at the Local Slaughterhouse in Khon Kaen, Thailand

Bongkot Noppon[1] Seri Khang –air[1] Prapansak Chaveerach[1] Pimsuree Ussawingowit[2] Apiradee Sopa[3]

[1] Department of Veterinary Public Health, Faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen, 40002, Thailand
[2] Master student for the Master of Science Program in Veterinary Public Health, Faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen, 40002, Thailand
[3] Research assistant for the Department of Veterinary Public Health, Faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen, 40002, Thailand

ABSTRACT

Objective: This study aimed at investigating the occurrence of *Salmonella* spp. at the local slaughterhouse in Khon Kaen province of Thailand.

Materials and Methods: Pooled samples were used in this study. The type of samples were carcass rinse waters, defeathering rinse waters, chilling waters, knifes, conveying belts, cages, buckets, chop board swab, before and after the operation of the slaughtering line. Aside from this, cecal content, breast meat, thigh, fillet, and chicken products such as chicken balls, steamed chicken in banana leaves, chicken sausages, and chicken bite were also collected. In total, 931 samples were collected during the 6 months interval, starting from September, 2012 to April, 2013. The ISO 6579:2002/AMD 1:2007 method was employed for culturing and identification of *Salmonella* species.

Results: The occurrence of *Salmonella* contamination was in the range of 33.93 -75.86%. The average occurrence of the 6 months interval was 52.50% (147/280). This indicated that the different flocks when come to slaughter, they were at greater risk of cross contamination since this study collected the earliest batch of birds during the day.

Conclusion: This study noted that there were high occurrence of *Salmonella* contamination at the slaughterhouse and control measure is warranted for the reduction of contamination during the slaughtering hours.

KEYWORDS: *chicken, occurrence, slaughterhouse, Salmonella*

Efficacy of Natural Thai Clays for Adsorption of Aflatoxin B1

Bundit Tengjaroenkul[1] Urai Tengjaroenkul[2] Nillapan Vongsahai[1]
[1] *Faculty of Veterinary Medicine, KhonKaen University, KhonKaen 40002, Thailand, btengjar@kku.ac.th*
[2] *Faculty of Science, Chiang Mai University, Chiang Mai 53200, Thailand*

ABSTRACT

Natural Thai clays from different areas and commercial adsorbents were investigated their adsorption capacities of aflatoxin B1 in vitro. The Thai clays were capable of sequestering AFB_1 from aqueous solution differently. The experimental data were fitted to modified Freundlich model for investigating adsorption capacities and affinity constant. The S-shaped isotherms studies to measure capacity and affinity of toxin adsorption were observed for these clays as well as the commercial adsorbents. Results of X-ray diffraction spectrometry demonstrated that several Thai clay contained mainly montmorillonite. These results may be useful for predicting the efficacy of adsorbents in vivo prior to include in animal feeds.

KEYWORDS: *Adsorbent, Contamination, Feed, Toxin*

Effects of Combined Mycotoxins between Aflatoxin B1 and Fumonisin B1 on Growth, Hematology and Antibody Titer against Streptococcal Vaccine of the Nile Tilapia Fish

Bundit Tengjaroenkul[1] Natapol Pumipuntu[2] Urai Tengjaroenkul[1] Piyawat Saipan[1]
[1] *KhonKaen University*
[2] *Mahasarakham University*

ABSTRACT

The objective of this research was to study the effects of combined toxins between aflatoxin and fumonisin contaminated in fish feed on growth, hematology immune response and histopathology of the Nile Tilapia. The experiment was divided into five treatments with three replications. Fish in treatment 1 was the control group fed a basal diet. Fish in treatment 2 was fed with aflatoxin B1 at 3 ppm (mg/ kg feed). Fish in treatment 3 was fed with aflatoxin B1 at 3 ppm and fumonisin B1 at 0.5 ppm. Fish in treatment 4 was fed with aflatoxin B1 at 3 ppm and fumonisin B1 at 1 ppm. And, fish in treatment 5 was fed with aflatoxin B1 at 3 ppm and fumonisin B1 at 2 ppm. The fish in treatment 2-5 were vaccinated intra-peritoneally with *Streptococcus agalactiae* vaccine, each 0.1 ml for 2 times at two weeks interval. After six weeks of the experiment, the results showed that fish weights of all experimental groups were lower when compared with the control group. The fish weight was decreased as the concentration of the toxin increased. Blood indices, including glucose, creatinine, lactate dehydrogenase, triglycerides and aspartate transaminase were increased significantly, while the levels of total protein, albumin and globulin were decreased statistically significant as compared with the control group. In addition, the mean level of antibody titer of the fish in each treatment against the streptococcal vaccine using direct agglutination test was decreased as the concentration of the toxin increased. And found that the effects of mycotoxins cause lesions in multiple organs, including liver and kidney. In conclusion, combined toxins between aflatoxin and fumonisin contaminated in fish feed can reduce growth, change levels of blood indices, decrease antibody titer against *S. agalactiae* vaccine and cause the histopathology effect in the Nile Tilapia.

KEYWORDS: *Toxin, Feed, Fish, Growth, Blood, Immune*

Analysis of Antibiotic Resistance among *Salmonella* Strains Isolated from Pig Origin in Vietnam

Luu Quynh Huong[1,*] Eric Jouy[2] Cédric Le Bar[3]

[1] *National Institute of Veterinary Research, 86 Truong Chinh road, Dong Da district, Hanoi, Vietnam*
[2] *Agence Française de Sécurité Sanitaire des Aliment, AFSSA Ploufragan- LERAP, Zoopole, Les Croix, 22440 Ploufragan, France*
[3] *Unité Animal et Gestion Intégrée des Risques (AGIRs), Centre de coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), Campus International de Baillarguet, TA C-22, 34398 Montpellier Cedex 5, France*
*Corresponding author: Department of Animal Hygiene, National Institute of Veterinary Research, 86 Truong Chinh road, Dong Da district, Hanoi, Vietnam, Tel: 00 84 91 4649774, Fax: 00 84 4 8694082
Email: lqhuongvet@yahoo.com

ABSTRACT

This study aimed to examine the susceptibility to 16 antimicrobial agents of a total of 102 Salmonella strains isolated from slaughter pig in Vietnam. No strain was found resistant to Amoxicillin clavulanic acid, Cefalexin, Ceftazidime, Cefotaxime, Ceftiofur nor Cefoxitin. 53% of the strains were resistant to at least one antibiotic, 48% of the strains were found resistant to Tetracycline, 39.2% to Sulfamide and 35.3% to Streptomycine. Multiresistance to six antibiotics (AM, TE, S, GM, SSS, TMP) was found for both *S*. Derby and *S*. Typhimurium.

KEYWORDS: *Salmonella*, *S*. Derby, *S*. Typhimurium, *Antibiotic, Antimicrobial resistance, slaughterhouse, pig, Vietnam*

Knowledge and Practice toward Nutritive Value and Safety Foods, and Food Sanitation of Food Employees

Patcharaporn Aree[1,*] Sukanya Parisunyakul[1] Suthatip Upalabut[1] Karnsinee Chanin[1]
[1] Faculty of Nursing, Chiang Mai University, Chiang Mai, Thailand 50200
*Corresponding author; Email: patcharaporn.a@nurse.cmu.ac.th

ABSTRACT

The aim of this study was to study food sanitation practice perceived by faculty members and student nurses and to determine an effect of advocacy nutritive value and safety program on knowledge and practice of food employees. Populations of the study were food employees, faculty members, and student nurses.16 employees and 47 faculty members and student nurses participated in this study. Research instruments were knowledge and practice questionnaires and food sanitation practice questionnaire.

The research finding were 1) the knowledge scores of the food employees at before and after intervention were 89.39(SD=5.86) and 90.26(SD=5.37), respectively. 2) The practice scores of the food employees at before and after intervention were 93.78(SD=5.93) and 93.56(SD=4.48), respectively. 3) The knowledge and practice scores of the food employees at after intervention were not significant differences than those at before intervention. 4) 9 of 10 activities of the food sanitation practices were graded at good level (approximately 50 %).

The results of this study provide the data that may be used for improving the food sanitation practices of food employees.

POSTER PRESENTATIONS
Emerging Infectious Vector Borne and Zoonotic Disease

A Study of the Occurrence of *Alaria alata* Mesocercariae in Pig Carcasses in Nine Provinces Bordering Mekong River, South of Vietnam

Duyen Thuy Thanh Phan[1,*] Lertrak Srikitjakarn[2,3] Saruda Tiwananthagorn[2] Phuong Thi Thuy Thai[4] Peter Paulsen[5]

[1] *Joint Master course in Veterinary Public Health (MVPH) of Freie Universitat Berlin and Chiang Mai University, Thailand*
[2] *Chiang Mai University, Thailand*
[3] *Veterinary Public Health Centre for Asia Pacific (VPHCAP), Faculty of Veterinary Medicine, Chiang Mai University*
[4] *Regional Animal Health Office No. 6, Vietnam*
[5]*University of Veterinary Medicine Vienna, Austria*
Corresponding author; Email: duyenphan1982@gmail.com

ABSTRACT

Alaria sp. is considered a potential zoonotic trematode, because an intermediate stage of this parasite has been implicated in human disease, although sporadically. As regards *A. alata*, final hosts of the parasite are (wild) carnivores. The intermediate hosts are wetland- or water-associated, e.g. snails and tadpoles/frogs, but a number of vertebrates can act as paratenic hosts of the mesocercarial stage of the parasite. From a historical perspective and considering food safety aspects, pigs have been identified as carriers of *Alaria* mesocercariae. These parasitic stages can be recovered from adipose tissue, salivary glands and skeletal muscles. *Alaria* spp. has been reported in America and Europe, but information from Asian countries is scarce. Based on the biology of *A. alata*, this study is taken as a pilot survey to investigate the occurrence of *A. alata* mesocercariae in fresh pork in Mekong Delta, South of Vietnam where the river density is high and the pig traditional farming has low bio-security. The samples were collected from pigs originating from the backyard farms at slaughterhouses in provinces bordering the branches of Mekong River from December 2012 to May 2013. The cheek and peritoneal tissue samples were collected from 621 pig carcasses immediately after slaughter process. The samples were tested by the "Alaria Migration Technique (AMT)" developed by Riehn *et al*. (2010). None of the samples tested positive, indicating that if *Alaria alata* is present in the population studied, the prevalence of infected pigs is less than 5% during the sampling period.

KEYWORDS: *Alaria mesocercariae, pig, Vietnam, Alaria-Migration-Technique*

Comparison of Bovine and Avian Purified Protein Derivatives on Bovine Tuberculosis in Chiang Mai Province (Thailand)

Tin Tin Lay[1,*] Anucha Sirimalaisuwan[2] Veerasak Punyapornwithaya[3]

[1] *LBVD, Ministry of Livestock Breeding and Fisheries, Myanmar and Master of Science (Veterinary Science), Faculty of Veterinary Medicine, Chiang Mai University, Thailand*

[2] *Department of Veterinary Bioscience and Veterinary Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Thailand*

[3] *Department of Food Animal Clinic, Faculty of Veterinary Medicine, Chiang Mai University, Thailand*

Corresponding author; Email: tintinlay7@gmail.com

ABSTRACT

Bovine tuberculosis (bTB), caused by *Mycobacterium bovis* (*M. bovis*) is one of the economically important zoonotic diseases. Single intradermal tuberculin test or comparative interdermal tuberculin test can be widely used for test and slaughter control program. In Thailand, Department of Livestock Development (DLD) annually performs the tuberculin test with bovine purified protein derivative (bovine PPD). In this study, we compared the cell mediated antibody responses of bovine and avian (PPDs) in dairy cattle. The study was conducted in dairy farms of Mae Wang district in Chiang Mai Province. Seventy four cows were tested with comparative intradermal tuberculin test (CIDT). Bovine PPD was injected at caudal region and avian PPD was injected at cervical region. Skin thickness were measured before and 72 hours after injection. For each injection site, the thickness of skin before and after injection was compared using paired T-test. The sizes of the skin thickness for bovine and avian PPD before and after injections and differences of each injection site were compared using Welch's T-test. Result showed that measurements of skin thickness before and after injections were significantly difference (P < 0.01). There was significant difference comparing the difference in skin thickness for each injection site (P < 0.01). At bovine injection site, 90% of tested cows showed $\leq$ 2mm skin swelling difference and 6.8% illustrated 3mm, 2.7% produced 4mm respectively. Likewise in avian injection site, 82% showed $\leq$ 3mm, 15.2% produced 4-7mm and 2.7% illustrated more than 9mm respectively.

KEYWORDS: *Bovine tuberculosis, PPD (Purified Protein Derivative), Skin thickness*

Effect of Dengue Virus Infection of Long-tailed Macaque (*Macaca fascicularis*) in Kosumpee Forest Park, Maha Sarakham Province

Captain Gerdsuwan[1] Chonruetei Sukma[1] Susadee Khemton[1] Natapol Pumipuntu[2,*] Pailin Jinagool[3] Worapol Aengwanich[4] Pornpit Vaisusuk[5]

[1] *Faculty of Veterinary Sciences, Mahasarakham University, Mahasarakham 44000, Thailand*
[2] *Department of Veterinary Public Health, Faculty of Veterinary Sciences, Mahasarakham University, Mahasarakham, Thailand*
[3] *Department of Veterinary Clinical Science, Faculty of Veterinary Sciences, Mahasarakham University, Mahasarakham, Thailand*
[4] *Department of Physiology, Faculty of Veterinary Sciences, Mahasarakham University, Mahasarakham, Thailand*
[5] *Veterinary Animal Hospital, Faculty of Veterinary Sciences, Mahasarakham University, Mahasarakham, Thailand*
Corresponding author; Email: film.natapol@gmail.com

ABSTRACT

The study of hematological values and effect of dengue virus infection in Long-tailed Macaques (*Macaca fascicularis*) were determined in Long-tailed Macaques at Kosumpee Forest Park, Maha Sarakham province. The blood samples were collected from cephalic vein and femoral vein of 30 macaques. Dengue virus antibodies detected by using a commercial test kit, the results of Dengue virus infection was found 6 infected macaques (20% of total samples) and 24 non-infected macaques (80% of total samples) show the incidence of Dengue virus infection in Long-tailed Macaques at Kosumpee Forest Park was occurred 1/5 of macaque population. Hematological values (mean ± SD) of non-infected macaques, founded WBC count 20438.89±5703.88 x 10^3cell/µl, Hemoglobin 13.04 ± 1.21 g/dL, Hemetocrit 39.67 ± 3.71%, Neutrophil 29.67 ± 13.76%, Lymphocyte 60.45 ± 14.29%, Monocyte 4 ± 2.44%, Eosinophil 5.89 ± 3.14 %, Platelet 300833.34 ± 103071.22 cell/µl, RBC count 5.05±0.39 x 10^6cell/µl, MCV 78.19 ± 2.12 fL, MCH 24.60 ± 5.02 pg, MCHC 32.16 ± 1.06 g/dL, was differenced in white blood cell count between infected and non-infected macaques ($P<0.05$). The results of this study can be used as a guideline for the prevention of disease from animals to humans.

KEYWORDS: *Dengue virus, Hematology, Long-tailed Macaques, Kosumpee Forest Park*

The Threats in the Making: the *Qurban*-Borne Diseases

Khalib Abdul Latiff[1,*]
[1] *Department of Community Health, Faculty of Medicine, Universiti Kebangsaan Malaysia*
Corresponding author; Email: khalib@ppukm.ukm.edu.my

ABSTRACT

Livestock slaughtering or *Qurban* is an annual ritual practice among Moslem. It is a must and required tradition during the peak "4 days" of the hajj season (10 - 13 Zulhijjah, the Hijri calendar) involving the main livestock such as cattle, goats, sheep and camels. As the demand is expected to be higher in time to come as the capacity to purchase is extremely huge, its related menaces are undeniable. Today, *Qurban* is not only limited among the locals but also the foreigner - as the workers (such as Indonesian, Bangladeshi, Indian and Bangsamoro), students (such as Yemen, Sudan, Libya, Somalia etc.) and even the Myanmar refugees (Rohingha). The scenario gets complicated as a massive influx of livestock from the neighbouring countries might involve the "sick" as well. The chance of cross-contamination from the abattoirs (the carriers), surrounding soil (the infected spores) and by-product of the animals (microbes) are also higher. Perhaps the most worrying is the diverse practices of Moslem from various culture background. Failure to adhere the hygienic principle can easily endanger the human life. In addition, the large numbers of illegal, undesignated and unhygienic abattoir houses might also pose a hazard if it is not properly cleaned or disinfested as it can be a good breeding places of various vectors (such as rodents, flies, mosquitoes etc.), the intermediate host of the common infectious diseases. To date, there is no uniformed precautious and response system being laid down on the ground to monitor and to tackle this possible health threats. Therefore a well planned mechanism is needed involving many parties among SEAHON members to prevent potential *Qurban*-borne Diseases in the future. As substantial number of population in this region are Moslem, a serious thinking and planning is extremely needed.

Sero-survey of Q Fever in Dairy Cattle in Chiang Mai Province, Thailand, 2012

Pranee Rodtian[1,*] Montri Nuamjit[1] Mongkol Srijun[2] Pattarin Opaschaitat[3] Monaya Ekgatat[3]

[1] *The Fifth Regional Livestock Office, Huay Kaew Road, Chiang Mai, Thailand, 50300*
[2] *Chiang Mai Provincial Livestock Office, Huay Kaew Road, Chiang Mai, Thailand, 50300*
[3] *National Institute of Animal Health, Kasetsartklang, Phaholyothin Road, Ladyao, Bangkok, Thailand, 10900*
Corresponding author; Email: pranee.rodtian@gmail.com, mobile phone: +668-1111-7097

ABSTRACT

Q fever is an emerging zoonosis in Thailand, caused by *Coxiella burnetii*. In 2011, a study in Khon Kaen focusing on zoonotic causes of endocarditis in humans identified four confirmed cases of endocarditis caused by Q fever. The purpose of this study was to estimate the proportion of cattle, an important reservoir of Q fever, with *C. Burnetii* antibody in Chiang Mai. Convenience samples of sera collected from dairy cattle in San Pa Tong, Mae Wang and Mae On Districts in Chiang Mai were analyzed by the National Institute of Animal Health using indirect ELISA. The proportion seropositive dairy cattle at herd and individual levels were 62% (13/21)and 5% (28/581),respectively. Mae On District had the highest proportion of seropositive dairy cattle in this study. No association was found between age, herd size and seropositivity. This result suggested that dairy cattle may be an important carrier of Q fever in farming communities and further investigation of Q fever burden in both livestock and farmers is warrant.

KEYWORDS: *Q fever, dairy cattle, Chiang Mai*

Expression and Characterization of Cathepsin D in Tsetse (*Glossina morsitans morsitans*)

Ruttayaporn Ngasaman[1] Zhou Mo[1] Shin-ichiroKawazu[1] Noboru Inoue[1]
[1] *National Research Center for protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan*

ABSTRACT

Background: Trypanosoma brucei and *Trypanosoma congolense* are causative agents of Human African Trypanosomosis (HAT) and Animal African Trypanosomosis (AAT), and have a strong socioeconomic impact in Sub-Saharan region. The transmission is related to the blood feeding insect vector, tsetse flies. Trypanosomes undergo complex life cycle development within tsetse fly, such as successive rounds of differentiation, proliferation and migration. In the fly midgut, blood stream form (BSF) trypanosomes rapidly differentiate to procyclic form (PCF). At the same time they must interact with the digestive enzymes and digestion products, which possibly modulate the parasite metabolism and its infectivity. However, the study in the dynamic of cathepsin D activity from the midgut of tsetse has not been reported.

Objective: The objective of this study is to characterize cathepsin D of tsetse and to investigate its role on tsetse-trypanosome interaction.

Methods: (1) The gene of *Glossina morsitans* cathepsin D (GmcathD) was cloned from the tsetse midgut. (2) The recombinant GmcathD (rGmcathD) was expressed by using *E. coli* and *Pichia pastoris* expression systems. (3) The enzymatic activity of the rGmcathD was analyzed by using general protein substrates (Hemoglobin: Hb and bovine serum albumin: BSA) and fluorogenic casein. (4) Analyze the effects of rGmcathD on trypanosome *in vitro*. (5) Tissue and cellular localization of GmcathD will be determined.

Results: Genes encoding aspartic acid protease named GmcathD was cloned from digestive tract of tsetse flies. The deduced amino acid sequence encoded one catalytic site (DTG), had predicted molecular mass of 28 kDa with PI value 7.1, and showed homology to cathepsin D-like aspartic protease of *Musca domestica* (50%). The enzymatically active rGmcathD was successfully expressed by *Pichia pastoris* expression system. The rGmcathD showed relatively high proteolytic activity toward fluorogenic casein at pH 6.0 at 37˚C. The rGmcathD digested common blood meal proteins (Hb and BSA) dose dependent manner. However, it preferred Hb than BSA as a substrate. The rGmcathD was inhibited specifically by the pepstatin.

Conclusion: This study revealed that Gmcath D showed catalytic activity at pH 6.0 that was close to the pH of endosome-lysosome compartment (pH 4-6), although other insect cathepsin D proteins had an optimal pH at 2.5-4.5. The results in this study also imply that yeast expression system is suitable for expressing recombinant tsetse proteases. Suggesting, the results and further studies may give basic interest and also lead to reference system for the identification of protease-mediated tsetse-trypanosome relationships.

The Contribution of Animals in Malaria Transmission in Nusa Tenggara Timur, Indonesia

Dewi Susanna[1] Tris Eryando[2]
[1] Department of Environmental Health, Faculty of Public Health, Universitas Indonesia
[2] Department of Population and Health Informatics, Faculty of Public Health, Universitas Indonesia
Corresponding author; Email: dsusanna2@yahoo.com, triseryando2@yahoo.com

Nusa Tenggara Timur is one of the provinces in Indonesia who has a high prevalence of malaria. The existence of animals around the house could contribute in transmission malaria. To understand the role of animals in malaria transmission, therefore, it is important to determine the risk of the existence of animals in village area of this province. A total of 38,000 households from the secondary data 'Basic Health Research' conducted by Ministry of Health 2007 analysed used Chi Square Test and Logistic Regression to calculate the risk of any size of animal in malaria transmission. The independence variables were the size of animals and the existence of cage, while the dependence variable was malaria itself. The size of the animals divided into four groups of animal, namely big animal (cow, horse and buffalo), medium animal (pig, sheep, and goat), small animal (cat, dog, and rabbit) and poultry (chicken, bird, and duck). The cage of the animals categorized into groups, they were exist and no exist. The result of chi square test showed that middle animals, small animals and poultry had p value less than 0.05, whereas big animals had no significant p value (p > 0.05). The existence the cage in the household also showed a risk in transmission with OR= 4.71 (3.63-6.10, p < 0.05). The multivariate logistic regression analysis revealed that all of big animals, middle animals, small animals, and poultry were the risk factors of malaria with OR 0.80 (0.72-0.89), 1.32 (1.19-1.44), 1.12 (1.03-1.22), and 1.158 (1.05-1.28) respectively. As a conclusion that big animals contributed as a protective factor to malaria, but middle animals, small animals, poultry and having a cage were a risk factor.

KEYWORDS: *animal, cage, malaria, poultry*

New Record of Bat Bugs Ectoparasite (Hemiptera: Cimicidae) from the Cave-dwelling Bats

Rutcharin Potiwat[1] Chamnarn Apiwathnasorn[1] Siriluck Attrapadung[1] Yudthana Samung[1]
Anon Payakkapol[1] Boonruam Chittsamart[2]
[1] *Medical Entomology Department, Faculty of Tropical Medicine, Mahidol University, Bangkok, 10400, Thailand*
[2] *The office of Diseases Prevention and control 2, Saraburi province, 18120, Thailand*

ABSTRACT

Bat bugs are blood-sucking insect parasites that feed primarily on the blood of bats and will also bite humans if given the opportunity. A bat bug is a small brownish insect, approximately half a centimeter in length, and having an ovular shape with a pronounced abdomen. Bed Bugs and Bat Bugs are classified in family Cimicidae (e.g. *Cimex lectularius*, *Cimex hemipterus*, *Cimex pipistrelli*, *Cimex pilosellus* and *Afrocimex constrictus*), which includes about 30 species distributed throughout the world. Both of Bed bugs and Bat Bugs are closely related and often very difficult to determine. Microscopic examination is needed to distinguish them by looking at the length of hairs on the upper covering of the thorax. Moreover, Bat bugs may move into human living areas and incidentally bite people, with such migrations particularly common when bats migrate or are carried with the traveler or backpacker. Bat bug bite symptoms typically appear as red, itchy welts but it has not been demonstrated to be effective transmitters of the disease. Our study are focus on identify hematophagous insect with suckling blood form the bat. The Cimicidae insect were collected manually with the aid of forceps, brushes and a magnifying glass, and fixed in 70% ethanol before clarify the species under microscope. The results from three cave-dwelling bats has been demonstrated the Cimicid Bugs (Hemiptera: Cimicidae) and only one cave that presented a new Bat bugs species, *Leptocimex inordinatus*. This species has not been recorded in Thailand.

Cobra Bites and *Shewanella* Infections

Po-Yu Liu[1,2] Zong-Yen Wu[1] Shu-Ying Tseng[1] Ching-Lin Shyu[1]
[1] *Department of Veterinary Medicine, College of Veterinary Medicine, National Chung-Hsing University, Taichung, Taiwan*
[2] *Department of Internal Medicine, Taichung Veterans General Hospital, Taichung, Taiwan*

ABSTRACT

Venomous snakes bite about 2.5 million people every year globally. South and Southeast Asia are the most affected regions and have the highest number of incidence and mortality due to snake bites in the world. The Chinese cobra (*Naja atra*) is the most medically important species in China and can cause severe local swelling and tissue necrosis. Bacterial infections may develop after snake bites but data on the incidence and bacteriology are limited. Human infections caused by *Shewanella* species are rare. Soft tissue infection is the most commonly described presentation. *Shewanella* infections after animal bites were rarely reported in the literature.

The study aims to investigate *Shewanella* wound infections following cobra bites and described the characteristics of these infections.

Medical records of patients with *Shewanella* wound infections after cobra bites presented to a tertiary medical center were reviewed. Clinical, laboratory, and microbiologic data were extracted from the medical charts of the patients.

Shewanella wound infection is severe complication after Cobra bite. Local reactions of envenomation were moderate to severe. Compartment syndrome may developed, in particularly when bitten in the upper limbs.

Cobra bites can cause extensive local damage. Infections secondary to cobra bites often result in rapid onset of cellulitis. The patients developed clinical signs of necrotizing skin and soft tissue infections included bullae and skin necrosis. The affinity of *Shewanella* for necrotic tissue has been reported. Wound infection following snakebite may be due to venom-induced tissue destruction, leaving it susceptible to *Shewanella* infection.

Shewanella Species in the Coastal Water and Aquaculture of Taiwan

Shu-Ying Tseng[1] Po-Yu Liu[1,2] Zong-Yen Wu[1] Ching-Lin Shyu[1]
[1] Department of Veterinary Medicine, College of Veterinary Medicine, National Chung-Hsing University, Taichung, Taiwan
[2] Department of Internal Medicine, Taichung Veterans General Hospital, Taichung, Taiwan

ABSTRACT

Shewanella is a facultatively anaerobic gram-negative bacilli. It is a saprophytic, marine organism which is often associated with opportunistic infections. Most reported cases had history of sea water exposure or seafood consumption. Recently, case reports of *Shewanella* infection have increased. Many of them were found in the Asian region and Taiwan.

The study aims to investigate the occurrence, distribution, and diversity of *Shewanella* spp. in Taiwan coastal water and mollusks.

Mollusks and overlying seawater samples were collected in the Taiwan coast. Samples were transported at ambient temperatures to the laboratory. In total, 80 mollusks samples and 25 water samples were collected. *Shewanella* was identified to species level using 16S rRNA sequences. More characteristics were analyzed using auxiliary biochemical, growth, and hemolytic tests.

A total of 64 *Shewanella* strains were isolated from aquaculture samples, included *S. haliotis, S. algae, S. chilikensis, S. marisflavi, S. aquimarina*, and *S. loihica*. 22 *Shewanella* strains were isolated from water samples, included *S.haliotis, S. algae, S. marisflavi, S. aquimarina, S. loihica, S. putrefaciens*, and *S. baltica*. The majority of *Shewanella* species amongst the *Shewanella* isolates were *S. algae, S. haliotis* and *S. putrefaciens*. Most *Shewanella* isolates are able to grow at 37°C, in the presence of 6.5% NaCl and exhibited hemolysis on sheep blood agar.

Further studies needed to identify its virulence factors and role in diseases.

The Emergence of Ocean Infectious Disease: *Shewanella*

Zong-Yen Wu[1] Po-Yu Liu[1,2] Ching-Lin Shyu[1] Shu-Ying Tseng[1] Shu-Peng Ho[1]
[1] Department of Veterinary Medicine, College of Veterinary Medicine, National Chung-Hsing University, Taichung, Taiwan
[2] Department of Internal Medicine, Taichung Veterans General Hospital, Taichung, Taiwan

ABSTRACT

The genus *Shewanella* are facultatively anaerobic, oxidase and catalase-positive, non-fermenter, Gram-negative, motile bacilli. They are widely distributed in marine and freshwater environments worldwide. Infections caused by *Shewanella* are uncommon but potentially fatal. Recently, case reports of severe *Shewanella* blood stream infection have increased. Many of them had underlying hepatobiliary diseases and were found in the Asia.

The objective of this study is to investigate the characteristics of severe *Shewanella* infection and its relation to environment.

This is a retrospective study conducted in a tertiary medical center in Taiwan. All known patients diagnosed with *Shewanella* were included. Clinical, laboratory, and microbiologic data were retrieved from the medical records of the patients.

Biliary tract infection and blood stream infection were important clinical presentations of *Shewanella* infections. Underlying hepatobiliary diseases including liver cirrhosis, hepatitis, biliary tract stones or hepatobiliary malignancy were important risk factors. *Shewanella* blood stream infection was the initial presentation of hepatobiliary malignancies in some cases, including cholangiocarcinoma, ampullary carcinoma and hepatoma. The mean sea surface temperature in the central coastal area of Taiwan is closely correlated with the cases of *Shewanella* infections in our study.

This association of *Shewanella* infections and mean sea surface temperature may reflect increased proliferation of *Shewanella* in warmer water, in the seafood, or both. Seasonal variation in the intestinal *Shewanella* of fish was also reported in other study. Adequate cooking kills *Shewanella*; however, seafood is often eaten raw or undercooked in Asian countries, thereby increasing the risk of infection by *Shewanella*.

POSTER PRESENTATIONS

EcoHealth - OneHealth System Strengthening

First Findings from an Assessment of Domestic Slaughterhouse Operations and Postmortem Inspection under the DLD Slaughterhouse and Butcher Shop Improvement Project in Livestock Region 1 of Thailand

Suphanan Boonyakarn[1,*] Veerasak Punyapornwithaya[2,3] Maximilian Baumann[4]

[1] Joint Master Course in Veterinary Public Health of Freie Universität Berlin and Chiang Mai University Thailand
[2] Department of Ruminant Clinic, Faculty of Veterinary Medicine, Chiang Mai University
[3] Veterinary Public Health Centre for Asia Pacific (VPHCAP), Faculty of Veterinary Medicine, Chiang Mai University
[4] International Animal Health, Faculty of Veterinary Medicine, Freie Universität Berlin, Germany
*Corresponding author; Email: suphanan63@yahoo.com

ABSTRACT

In 2010, the Department of Livestock Development (DLD) set up the "Slaughterhouse and Butcher Shop Improvement Project" to help small and medium sized slaughterhouses and butcher shops which intended to upgrade their slaughter- and butcher facilities to become standardized in order to protect consumers better in terms of food safety and to provide enhanced support for meat inspectors to perform ante-mortem and post mortem inspection. The objectives of this cross-sectional study were to assess and monitor pig and poultry slaughter conditions, facilities for meat inspection and to record post-mortem inspection. A questionnaire and recording form were developed based on the DLD meat inspection form and on the new regulation "Ministry of Agriculture and Cooperative's Regulation on Determination of Criteria, Procedures and Conditions for Establishing the Slaughterhouse, Lairage and Animal Slaughter B.E. 2555 (2012)". The study was conducted during January – April 2013 in 5 selected slaughterhouses being 2 medium size pig slaughterhouses, 1 large size and 2 small size chicken slaughterhouses in Livestock Region 1 of Thailand. The post-mortem inspection facilities are not sufficient in terms of specific location, area space, suitability to inspect, availability of light and provision of hand washing by tap and/or hose. Post-mortem inspections were recorded for 30,195 chickens and 1,352 pigs slaughtered. For the pigs inspected, lung lesions and pleuritis were the most common lesion with 59.9%, followed by skin lesion with 14.6% and lesions at liver, spleen, heart and lymph nodes with 14%, 8.4%, 7.5% and 7.3%, respectively. The lesions found in chicken were 3.6% bruises/fractures, 1.3% arthritis, 1.2% skin lesions, 1.1% over-scalded and 0.3% was dead on arrival.

KEYWORDS: *Thai domestic slaughterhouse, Post-mortem meat inspection, Livestock Region 1*

Outcome Mapping as a Monitoring and Evaluation Tool in the Ecohealth Field Building Initiative Leadership Initiative (FBLI) in South East Asia

Giang Thi Huong Pham[1] Tung Xuan Dinh[2] Hein Mallee[3] Hung Viet Nguyen[4]

[1] Center for Public Health and Ecosystem Research (CENPHER), Hanoi School of Public Health (HSPH) & Vietnam Public Health Association (VPHA)

[2] Vietnam National Institute of Animal Science

[3] Research Institute for Humanity and Nature

[4] Center for Public Health and Ecosystem Research (CENPHER), Hanoi School of Public Health (HSPH); Swiss Tropical and Public Health (Swiss TPH) & International Livestock Research Institute (ILRI)

ABSTRACT

Monitoring and Evaluation (M&E) activity is essential in any project management and helps to verify if a project has met the planned activities, outputs and outcomes. We present here M&E framework for the Ecohealth Field Building Initiative Leadership Initiative (FBLI) in South East Asia using Outcome Harvesting as a tool. Outcome Harvesting as a variation of Outcome Mapping - a M&E concept focusing on one specific type of result: outcomes as behavioral change - has been used to monitor and evaluate the achievements of hundreds of networks, non-governmental organizations, research centers, think tanks, and community-based organizations around the world.

We will describe the process of monitoring used in the FBLI program i.e. how proposed tools such as detailed monitoring and the quarterly bulletin, the self-reflection tool, as well as Outcome Harvesting are integrated to monitor and evaluate the expected outputs as well as what the program's varying hopes and expectations are, laid out in the project proposal as what the program would love to see, like to see and expect to see in the field of Ecohealth building in South East Asia.

The five year FBLI program, funded by the IDRC, is a research project aimed at positing Ecohealth as a sustainable dynamic field in the region. The three major components of the project, namely capacity building, knowledge translation, and research, are the progress markers that can be considered as indicators of behavioral change in boundary partners as well as desired outcomes in the Ecohealth field. These provide a basis for monitoring the progress and outcomes of the program.

Human-Animal-Ecosystem Mix: Keys to Risky New Living Style

Khalib Abdul Latiff[1,*]

[1] *Human Behaviour Consultant, Department of Community Health, Faculty of Medicine, Universiti Kebangsaan Malaysia*
Corresponding author; Email: khalib@ppukm.ukm.edu.my

ABSTRACT

As citizen are more urbanized and life so modernized and advance, the demand for domestic and wild animals for life is unavoidable whether for food, spiritual, body accessories, recreational or even companionship. The trend of human-animal interaction is getting more complex, creating new behaviour, new living style and thus resulting the new public health risks. The threats are not necessarily confined to food and food-related activities while handling and/or using the animals, but also other issues that are related to non-food matters as well. Evidences have shown that both zoonotic and non-zoonotic diseases are continuously reported and in increasing trend all over the globe as a result human-animal-ecosystem interface. However, the emerging and re-emerging of new living styles related to that are not given a great concern although it is known to be the source of the menace. This paper highlights some of proven living styles involving human-animal-ecosystem mix and its related health consequences that need be focused when devising effort for prevention and controlling public health risks.

Evaluation on Implementation of Good Farming Practices (GFP) and Good Hygienic Practices (GHP) in "Jaya Abadi" Milk Cooperative, East Java, Indonesia

Maria Angelina Puspitasari[1] Rarah Ratih Adjie Maheswari[1] Epi Taufik[1]

[1] Faculty of Animal Science, Bogor Agricultural University, Bogor, Indonesia 16680

ABSTRACT

Milk is a perishable food commodity due to its nutrient content. The perishability of raw milk, should encourage farmers as milk producers to constantly apply Good Farming Practices (GFP) and Good Hygienic Practices (GHP) in their farms. The study to evaluate the implementation of GFP and GHP in the dairy farms was done through interview and observation by using questionnaire to 29 sample farmers of "Jaya Abadi" milk cooperative member. This cooperative is one of the raw milk suppliers for Nestle Indonesia Co. The questionnaire was constructed based on "Guide to good farming practices for animal production food safety" (OIE, 2006) and "Guide to good dairy farming practice" (IDF-FAO, 2004). Five important aspects of GFP and GHP have been evaluated as follows: (a) farm building and facilities, (b) feed management, (c) human resources, (d) milking management, (e) farm management. The results showed that the majority of the farmers (87.76%) had less implemented GFP and GHP aspects in their dairy farming practices. This condition was positively associated with low microbiological quality of the milk, based on total plate count (TPC) analysis results of Nestle Indonesia Co.

Achieving Food Safety, the Improvement of Small-scale Slaughterhouses through Policy Engagement

Suwit Chotinun[1] Suvichai Rojanasthien[1] Fred Unger[2] Manat Suwan[3]

[1] Faculty of Veterinary Medicine, Chiang Mai University
[2] International Livestock Research Institute
[3] Faculty of Social Sciences, Chiang Mai University
Corresponding author; Email: suwitchotinun@gmail.com

ABSTRACT

Food safety concerns are important to consumers and a focus of the Thai government. Applying food safety standards is often challenging for poultry production especially in rural area. Previous studies indicated that poultry meat and meat products were highly contaminated with food-borne pathogens including *Salmonella* and *Campylobacter* with poultry slaughterhouses as a major source for bacterial contamination. There is a lack of studies targeting effective interventions to improve poultry meat production and hygiene, particularly on policy that regulates such interventions. Using an EcoHealth approach, this study, aimed to develop feasible and cost effective guidelines for improvement of small-scale poultry slaughterhouses in Northern Thailand. Policy makers were involved from the early stage. Current laws and regulations for poultry slaughterhouses were reviewed, in depth interviews and focus group discussions with district and provincial authorities provided information on the enforcement of those regulations. Forty-one small-scale poultry slaughterhouses located in Chiang Mai were visited during July 2011-February 2013. Data on the current management and the need for improvement to address the current regulations were collected from slaughterhouse owners using structured questionnaires and checklists. The study revealed that despite the enforcement of slaughterhouse regulation since 1992 and GMP since 2006, none of the small-scale slaughterhouses in this study are able to meet the regulations and obtain license. The slaughterhouse owners reflected that current regulations and GMP are not feasible for small-scale slaughterhouses. Improper hygienic management was commonly practiced. The guideline for improving the slaughterhouse is developed and currently being implemented and evaluated through outcome mapping in selected slaughterhouses. Results of this study were discussed in a policy meeting with policy-level officers and will be reflected in a policy brief. Feedback from those discussions will be presented.

KEYWORDS: *Small scale poultry slaughterhouse, food safety, policy engagement*

A Social Network Analysis of Simulated Backyard Chicken Trade during Chinese New Year Festival in Phitsanulok Province, Thailand: Implications on HPAI H5N1 Spread

Anuwat Wiratsudakul[1,2,*] Mathilde C. Paul[3,4] Thanawat Tiensin[5] Wannapong Triampo[6] Karine Chalvet-Monfray[1]

[1] INRA, UR346 d'Epidémiologie Animale/Université de Lyon Vet Agro Sup Campus Vétérinaire de Lyon, Marcy-l'Étoile, France
[2] The Monitoring and Surveillance Center for Zoonotic Diseases in Wildlife and Exotic Animals,
Faculty of Veterinary Science, Mahidol University, Nakhon Pathom, Thailand
[3] INRA, UMR 1225 IHAP/Ecole Nationale Vétérinaire de Toulouse, Toulouse, France
[4] Université de Toulouse, INP, Ecole Nationale Vétérinaire de Toulouse, Toulouse, France
[5] Division of Livestock Foreign Affairs, Department of Livestock Development, Bangkok, Thailand
[6] Institute for Innovative Learning and Department of Physics, Faculty of Science, Mahidol University, Bangkok, Thailand
*Corresponding author: Université de Lyon Vet Agro Sup Campus Vétérinaire de Lyon, 1 Avenue Bourgelat, 69280 Marcy-l'Étoile, France. Tel.: +33 4 78 87 26 68; Fax: +33 4 78 87 27 93; Email: anuwat.wiratsudakul@vetagro-sup.fr

ABSTRACT

Highly Pathogenic Avian Influenza H5N1 (HPAI H5N1) is a public health threat and an economically important disease which affected Thailand during the last decade. The virus was mainly detected in backyard chicken population during epidemics. Movement and trade of live chicken played an important role in virus spread. This study aimed to compare backyard chicken trading activities during the Chinese New Year festival (day 17-31 of the year) and regular time (day 335-349). It included chicken census data collected by veterinary services, as well as data on trading activities collected on the field from 2009 to 2012. We initially used a compartmentalized stochastic dynamic model to simulate backyard chicken trading patterns for different types of chicken collectors including household collector, collector slaughterhouse and collector-of-collector. We took into account the higher demands of chickens during the Chinese New Year festival. The simulated outputs were then analyzed as symmetric weighted two-mode networks. The first mode was the villages located in Phitsanulok province and the second mode was chicken collectors. Packages for graphs and social network analysis so-called 'igraph' and 'tnet' in statistical language program R were used for analyzing the social network parameters. Our results revealed that the network backyard chicken trade during Chinese New Year festival contained 384 villages with 605 connections, while the network of regular time had 316 villages with only 418 connections. An average weighted degree centrality of Chinese New Year festival was 5.0 (range: 1-149) and it was 4.7 (range: 1-138) in the regular period. We found average K-core of 1.7 (range: 1-4) and 1.4 (range: 1-3) in Chinese New Year festival and regular period respectively. It was concluded in this study that trading activities of backyard chicken during Chinese New Year festival were significantly higher than in regular time (for weighted degree centrality and for K-core p-value < 0.001). Thus, movement and trade of backyard chicken during festive period could cause wide spread of the outbreak and we should closely monitor and strengthen HPAI H5N1 surveillance program during Chinese New Year.

KEYWORDS: *backyard chicken, Chinese New Year, avian influenza, modelling, social network analysis, Thailand*

A Participatory Ecohealth Study of Smallholder Pig System in Upland and Lowland of Lao PDR

Inthavong Phouth[1] Durr Peter[2] Khamlome Boualam[3] Blaszak Kate[1,2] Somoulay Virasack[4] Allen John[2] Gilbert Jeff[5]

[1] National Animal Health Centre, Vientiane, Laos
[2] Australian Animal Health Laboratory, Geelong, Australia
[3] Department of Hygiene and Prevention, Vientiane, Laos
[4] National Centre for Laboratory and Epidemiology, Vientiane, Laos
[5] International Livestock Research Institute, Vientiane, Laos

ABSTRACT

A cross-sectional study to determine baseline seroprevalence of key pig zoonoses and some of priority pig production diseases and to evaluate public health risks of pig-raising and pork consumption in one upland and one lowland province, Lao PDR. The surveys were conducted in two provinces, Louangphrabang representative of 'upland' and Savannaket province for 'lowland'. The selection of villages is weighted by village human population derived from the 2005 National census and GIS data. Participatory questionnaire development and data storage was enabled by a new web based programme called SurVet. Humans were tested for exposure to Taenia/Cysticercosis, Trichinellosis, Hepatitis E virus and Japanese encephalitis virus (pigs: Trichinella, HEV, JEV, Erysipelas, CSF, FMD and PRRS). The training and field activities were done with integrated transdisciplinary approaches involving district and provincial staff, as well as students.

Result: Provisional seroprevalence and relevant odds ratios show that the viral diseases Hepatitis E and Japanese Encephalitis are widespread in both provinces, but that the parasitic diseases cysticercosis and trichinellosis are more sporadic, the latter apparently associated more with educated males of certain ethnicities. There are also results pertaining to pig health diseases and associated management risks. Further potential for multivariate statistical analysis exists.

Outputs: Through the identification of the spatial patterning of seroprevalence and risk factors associated with exposure to these diseases, the IEC materials such as posters and brochures on human health and animal health risk reduction have been developed and produced as well as guide future research and policy

Rapid Integrated Assessment of Food Safety Related to Pork in Vietnam: A Consumer Perspective

Nguyen Viet Hung[1,2] Luu Quoc Toan[1] Dang Xuan Sinh[1] Nguyen Tien Thanh[1] Pham Van Hung[3]
Delia Grace[2]

[1] *Center for Public Health and Ecosystem Research (CENPHER), Hanoi School of Public Health, Hanoi, Vietnam*
[2] *International Livestock Research Institute (ILRI), Hanoi, Vietnam and Nairobi, Kenya*
[3] *Department of Quantitative Analysis, Faculty of Economics and Rural Development, Hanoi University of Agriculture, Vietnam*

ABSTRACT

In Vietnam, pork makes up 75% of meat consumed, with its production delivering substantial benefits to the smallholders who supply 84% of the market. However, pork contains high levels of pathogens, an issue of growing concern among the public, and policy makers alike. To respond to these concerns, we developed a rapid integrated assessment tool with partners to assess food safety and zoonosis related to pork value chain and tested it in different countries. This study presents the results of this rapid assessment of food safety and zoonosis from a consumer perspective and with analysis of biological samples.

Focus Group Discussions (FGD) were conducted in Hung Yen and Nghe An provinces for regular pork consumers and pregnant women or mothers of young children. In each province, three districts were selected, and one commune selected per district. Six FGD of 7 persons, stratified by income, were conducted in each commune, giving a total of 36 FGD and 252 participants in two provinces. The FGDs focused on food safety (hazard and risk), nutrition, and social and gender determinants of health and nutrition. Eighty pork samples were also collected in two province to rapidly capture the quality of pork from slaughterhouse and market through the measurement Total bacterial count (TBC), coliform, Water Holding Capacity (WHC) and pH.

Participants were housewifes who often buy food for their family and are in charge of cooking pork. Pork is the main meat eaten daily in both provinces, representing 50-60% of total ASF consumption. Meat was bough mainly from the informal market and quickly prepared, cooked and consumed. People had high trust in pork safety and quality and rarely attributed health issues to pork consumption. Raw pork is rarely eaten except for fermented pork (nemchua) which is occasionally consumed. The main concern was growth promoters, pork refresher (chemicals used to make not fresh pork appear fresh) as well as diseased pork. There was little knowledge of zoonoses. The pork portions perceived as rich in nutrients were used young children and special care was given to their preparation, such as cooking well or making into soup.

The TCB in swab samples at slaughterhouse varied from 10,500 to 3,410,000 CFU/400cm^2 and coliform from 20 to $1.1*10^4$ MPN/400cm^2 .Pork samples collected in the market had TCB varying from $4.3*10^4$ to $3280*10^4$ CFU/g and coliform 40 to $1.1*10^4$ MPN/g Pork pH after 1, 4 and 6h after slaughtering was in the range of 6.07-7.00), 5.35-6.95 5.15-6.02 respectively. The drip loss test showed that the water loss was from 1.26 to 5.92% after 48h.

In conclusion, this rapid assessment shows that meat is a main animal food source in Vietnam and women are responsible for buying and preparing pork. While the trust in pork quality was high, microbial and physio-chemical analyses suggest further studies to address consumers' concern on chemical contamination.

KEYWORDS: *Vietnam, pork, rapid assessment, food safety*

One Health – EcoHealth in Vietnam

Nguyen Viet Hung[1] Phuc Duc Pham[1] Tung Xuan Dinh[2] ToanQuoc Luu[1] GiangThiHuong Pham[1]

[1] *Center for Public Health and Ecosystem Research (CENPHER), Hanoi School of Public Health*

[2] *Vietnam National Institute of Animal Science*

ABSTRACT

One Health and Ecohealth have been the key approaches not only in controlling diseases and bringing resources from human and animal health sectors but also in mobilizing the participation of many stakeholders from varied sectors. Currently, communication and exchange between One Health and Ecohealth activities in Vietnam are not occurring frequently. Therefore, we believe that gathering all forces from all the programs, projects and initiatives of One Health and Ecohealth in the country would have greater impact in improving human, animal in improving human, animal, environmental health. In this view, we proposed creating a platform for exchanging information on networking, training, research, and advocacy between One Health and Ecohealth practitioners in Vietnam to improve the operations of both fields

A quarterly brief called One Health-Ecohealth in Vietnam consists of a focus section, activity highlights, and upcoming events related to One Health and Ecohealth in Vietnam. The core group that initiated the platform comprises of people from universities, research institutes, intergovernmental organization, ministries, and international organizations in Vietnam, especially the Ecohealth Field Building Leadership Initiative(FBLI) in South East Asia project (funded by the IDRC, Canada) and Vietnam One Health University Network (VOHUN, funded by the USAID).

KEYWORDS: *One Health, Ecohealth, the FBLI, the VOHUN, animal health, human health*

Health Risk Analysis of Benzene Exposure at Employees at 'X' Gas Station in Pancoranmas Depok 2011

Robiana Modjo[1,*] Rendy Noor Salim[1] Julia Rantetampang[1] Tri Mulyani[1]
[1] Department of Occupational Health and Safety, Faculty of Public Health, University of Indonesia
Corresponding author; Email: 71.bian@gmail.com

ABSTRACT

Benzene is one of the gasoline compounds which become a major concern to cause health problems. Petrol operators in gas filling point are the population at high risk to get exposed with benzene, especially through inhalation in continuous exposure. *Agency for Toxic Substances and Disease Register* estimated that the average exposure to benzene to the workers at gas station area was 0.12 ppm. High level exposure to benzene through inhalation can cause death. Thus, the low dose exposure chronically can cause dizziness, fast heartbeat, headache, tremor, confusion and easily distracted.

These research objectives were to explain the source and exposure intake, risk level of benzene exposure related to the worker's health risk (carcinogenic and non-carcinogenic) at The X Gas Station (SPBU) in Pancoranmas Depok.

In total, the respondents equal as the total population were 15 workers, consisting of 13 petrol operators in filling point and 2 administration workers. Data of benzene concentration in the air were obtained by direct measurement in December 2011 using Coconut shell charcoal refers to the NIOSH method 1501, 1994 and analyzed with Gas Chromatography (GC). Risk analysis was based on risk management and risk communication.

The study showed that the individual calculation of non-carcinogenic effects in real time exposure for 3 years and lifetime exposure to benzene was no risk to non-carcinogenic effects ($RQ \leq 1$). In contrast, the individual carcinogenic calculation indicated that in the 3 years of exposure, there was 1 employee at risk of cancer (ECR>10-4) while the employees were at risk of cancer to life time exposure.

In initial conclusion, we suggested that risk controls should be implemented, such as administrative controls and personal protective equipment (PPE). For the PPE, it is supposed to be half mask with organic vapor cartridge respirator as appropriate PPE to minimize exposure to inhaled benzene in the air.

KEYWORDS: *Health risk analysis, benzene, exposure, carcinogenic, non-carcinogenic*

EcoHealth Research in South East Asia: Past, Now, and the Ways Forward

Nguyen Viet Hung[1,2] Jeff Gilbert[2] Dinh Xuan Tung[3] Hein Mallee[4] Fred Unger[2] Delia Grace[2]

[1] *CENPHER, Hanoi School of Public Health, Hanoi, Vietnam*

[2] *International Livestock Research Institute (ILRI), Nairobi, Kenya*

[3] *Nantional Institute of Animal Sciences, Hanoi, Vietnam*

[4] *Research Institute for Humanity and Nature, Kyoto, Japan*

ABSTRACT

EcoHealth is one of the comprehensive concepts to look at health as an integrative component of the complex relation of human, animal and environment. Although it was introduced in South East Asia (SEA) late in 2000's by IDRC, its development in the region shows a dynamics in the landscape of research and application of EcoHealth in various fields such as emerging and zoonotic diseases, agriculture and health, education and training. The objective of this presentation is to review EcoHealth activities in SEA of the last 10 years to address lessons learned, challenges and future of EcoHealth in the region.

We analysed all the EcoHealth programmes, initiatives and projects (now called projects) that have been being implemented in the past 10 years with support of IDRC in SEA. Main considered EcoHealth projects are: APEIR (Asian Partnership on Emerging Infectious Diseases Research), EcoZD (Ecosystem Approaches to the Better Management of Zoonotic Emerging Infectious Diseases in SEA), EcoEID (EcoHealth emerging infectious diseases research in SEA), FBLI (Field Building Leadership Initiative in SEA), BECA (Building Capacity in EcoHealth for SEA). The level of Ecohealth characterised by how much "EcoHealth content" was analysed.

The results showed that generally, EcoHealth has been well perceived and taken by partners, in particular academia, policy makers and communities and generated some good research results in the field of ZEIDs. Some projects focused purely on capacity, others on research or both. However, the challenges remain at the project design and implementation level but also on the available capacity and coordination to develop EcoHealth research and teams in the countries as well as the issue of EcoHealth scaling-up. Finally we will present the ways forward of EcoHealth from a regional perspective in terms of research, training and policy translation using EcoHealth in combination with One Health approach.

KEYWORDS: *EcoHealth, South East Asia, (z)EIDs, interdisciplinary, scientific partnership, capacity building, network, EcoHealth content*

Chikungunya as an Neglected Tropical Disease in Travelers Kecamatan Bayah, Lebak

Indang Trihandini Trihandini[1]
[1] SPH UI

ABSTRACT

Chikungunya is a viral disease and it causes fever and severe joint pains share some clinical signs with dengue, the virus is transmitted from human to human by the bites of infected female mosquitoes (*Aedes aegypti* and *Aedes albopictus*). These mosquitoes can be found biting throughout daylight hours, although there may be peaks of activity in the early morning and late afternoon. However in 2010, Chikungunya disease attacked 163 people in five villages in the district Bayah, Lebak and the health district office term it as a Disease outbreak (KLB). Bayah (Lebak) has one of famous beaches, Sawarna as a favorite place for international or domestic travelers. In Addition, Pan American Health Organization, 2011 had been reported that Viremic travelers also spread outbreaks from India to the Andaman and Nicobar Islands, Sri Lanka, the Maldives, Singapore, Malaysia, Indonesia. In 2010, imported cases also were identified in Taiwan, France, and the United States. These cases were infected viremic travelers returning from Indonesia, La Réunion, and India, respectively. The study was conducted to measure the knowledge of Chikungunya disease among people who live in Sawarna. 115 people had been selected by random. The study design was cross sectional. The results are 75 % had known Chikungunya symptoms, but only few had known how to spread and prevent of Chikungunya. The result found also that the recording and reporting in the surveillance system is weak

KEYWORDS: *Chikungunya disease, viremic travelers, Neglected Tropical Disease*

An Integrative Approach to Elucidate and Enhance Hygienic Practices in Small-scale Poultry Slaughterhouses in Northern Thailand

Suwit Chotinun[1] Suvichai Rojanasthien[1] Fred Unger[2] Manat Suwan[3]
[1]*Faculty of Veterinary Medicine, Chiang Mai University*
[2] *International Livestock Research Institute*
[3] *Faculty of Social Sciences, Chiang Mai University*
Corresponding author; Email: suwitchotinun@gmail.com

ABSTRACT

In Thailand small scale rural poultry production development can be challenged by food safety policies that limit economic development opportunities. This may limit incentives for improving sanitation and disease prevention. Therefore, the project funded by the International Development Research Centre and implemented by International Livestock Research Development was performed. In this study integrative research including a participatory approach was used to elucidate and enhance the hygienic practices in small scale poultry slaughterhouses in rural Northern Thailand. Initial steps included the identification of stakeholders associated with meat production chain, development of a research framework, and designing the methodology based on stakeholder consultations. The framework and methodology derived combined at least five issue areas corresponding to the following disciplines1) public health 2) socio-economic 3) policy 4) veterinary and 5) community and environment. Methods used were questionnaires, observation, focus groups, and in-depth interviews. In addition, a microbiological risk assessment approach was employed to identify hazards and critical factors of slaughtering process affecting food safety and emerging food born pathogen risk. Combining this with stakeholder knowledge and attitudes provided the basis for identifying feasible and sustainable interventions.

This study revealed that there were complex factors affecting to the hygienic management of the slaughterhouse. The study also demonstrated the potential of an integrative, participatory approach for addressing a critical problem at the interface of rural development and public health. It may serve as a useful model as basis for study and intervention for other similar transdisciplinary challenges.

KEYWORDS: *Integrative approach, hygienic management, small-scale slaughterhouse, Northern Thailand*