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Original article

FOOD SAFETY CONCEPTS AND FOODBORNE BACTERIA IN FOOD ANIMALS IN THAILAND, LAO PDR AND VIETNAM

Pawin Padungtod,[1] Mutsuyo Kadohira,[2] Glen Hill [2]

[1]*Faculty of Veterinary Medicine,Chiang Mai University*
[2]*School of Agriculture, Obihiro University of Agriculture and Veterinary Medicine Obihiro, Japan*

Abstract Apart from sharing the Mekong river, Thailand, Vietnam and Lao PDR also have in common some geographical characteristics and traditions including food preparation. Food from animal origin such as chicken and pork are often contaminated with Salmonella or Campylobacter and sometimes consumed uncooked. These bacteria present the risk of severe gastrointestinal and other diseases locally and in countries that import such food. Recently, the concept of risk analysis has been applied to food safety; in particular, this risk assessment is comprised of hazard identification, exposure assessment, hazard characterization and risk characterization. Some strains of these foodborne bacteria cause diseases in animals that serve as food sources; however, public health problems usually arise from human pathogenic strains, which do not cause disease in animals. In these countries, *Campylobacter* was the most frequently isolated bacteria in children with foodborne gastrointestinal disease followed by *Salmonella, Shigella* and *E.coli. Salmonella* and *Campylobacter* were found in pigs and chickens at farms, slaughterhouses and markets. Furthermore, some of these bacteria may be resistant to various antimicrobial agents as a result of subtherapeutic use of these agents in livestock production. Therefore, infection by these resistant bacteria may be difficult to treat in patients who require antimicrobial treatment. Recent molecular epidemiological studies showed that these bacteria may be transmitted from food animals to humans through food processing procedures, and genes conferring antimicrobial resistance may be transmitted to humans and to other pathogenic bacteria via mobile genetic elements, presenting the potential to spread across the globe. Surveillance and collaborative research among these three countries may clarify the epidemiology of foodborne infections common in the regions. The results will also facilitate control of the bacterial contamination in livestock and food of animal origin, thus reducing the risk of foodborne diseases in the region and countries which import these food products. **Chiang Mai Veterinary Journal 2007;5(2):113-122**

Keywords: food safety , foodborne bacteria , food animal, risk assessment

Thailand is located in Southeast Asia, slightly to the north of the equator. It borders with Myanmar on the west, Laos PDR on the east, and Malaysia in the south. Thailand shares the Mekong River with other countries, in what is called the Greater Mekong Region (GMS). Mekong river begins in southern China and runs along the borders of Myanmar, Thailand, Laos PDR, Cambodia, and Vietnam, then cuts through Vietnam and drains into the South China Sea.

Address request for reprints: Pawin Padungtod, Department of Veterinary Public Health,Faculty of Veterinary Medicine, Chiang Mai University , Chiang Mai 50100, Thailand ; Email: padungto@chiangmai.ac.th
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Table 1. Demographical characteristics of Thailand, Vietnam and Laos PDR[1]

	Land area (1,000 sq.km.)	Population (million)	Density (persons/sq.km.)
Thailand	511.8	64.9	126.7
Lao PDR	230.8	6.1	26.4
Vietnam	325.4	82.7	254.1

Table 1 shows some demographical characteristics of these countries. Thailand is the largest country in this study, with more than 500,000 square kilometers of land, followed by Vietnam and Laos PDR. However, Vietnam is more heavily populated, so it has higher population density than Thailand[1]. The majority of the population in these countries are farmers. Rice is the main carbohydrate source, and Buddhism is the predominant religion. Resembling geographical and social characteristic lead to similar food source, cooking and eating preference among these countries.

In these countries, there are some department stores where meat and other fresh produces are kept refrigerated; however, the majority of meat and fresh produce are sold in open air markets with no refrigeration, especially in rural areas. In the city such as Bangkok, up to 70% of the people do not cook their own food, but instead they buy ready to eat food from open markets. Although meats are commonly cooked by grilling, roasting, or boiling, the prepared food is usually left unheated for quite some time before being sold and consumed. The microbial hazard in the food (either fresh or cooked) may easily increase in the tropical climate in these countries. Worst of all, some of the meat preparations are commonly consumed uncooked, such as *Nham* ,a fermented pork sausage popular in every countries. In some areas of Thailand, people also consume raw minced beef or pork when drinking alcohol. These factors facilitate transmission of microbial hazard to the consumer through food particularly food from animal origin.

Therefore, in this paper, we would like to review food safety concepts and situations focused on major foodborne bacteria and discuss how to improve food hygiene in Thailand, Lao PDR and Vietnam

Modern concepts of food safety

Nowadays, the impact of food on health has become an interesting issue for the general public around the world. The food we eat may no longer be produced locally or even domestically. Raw material such as meat and vegetable may be produced in a country on one continent then transported to another continent for further processing and consumption. For example, yakitori (grilled chicken) sold in Japan may be produced in Thailand or Brazil, cheese sold in Thailand may be produced in Europe, and fruits and vegetables sold in the USA may be produced in South American countries. This transcontinental food trade may increase the economic efficiency of food production, but it also open doors to transcontinental disease transmission. Because the transcontinental food chain involves more steps and longer time from production to consumption, the probability of contamination and the amount of microbiological hazard from microorganism and chemical hazards from their toxin in the ready to eat food increase. These hazards may present dangers such as foodborne diseases in consumers.

The probability of adverse outcome can be called a risk. The important characteristic of risk is the uncertainty of the occurrence, timing and magnitude [2]. For example, the occurrence of diarrhea in those who consume raw pork is uncertain because not everyone who consumes the pork may become ill. For those who become ill, they may not be ill at the same time, and the severity may vary among the patients. Therefore, we may refer to this event as the risk of diarrhea from consumption of raw pork. In epidemiological terms, specifying the risk of disease within a period of time is referred to as cumulative incidence. The risk of foodborne disease may be the result of contamination at any point from the production of livestock at the farm to consumption of ready to eat food by the consumers. Food animals may be infected with foodborne pathogens at the farm, most of which do not cause disease in animals. Then, the contaminated carcasses derived from infected animals may cross-contaminate other carcasses during slaughter and processing through the equipment, environment or workers. Microbial organisms may increase in number during storage at the market. Cross-contamination among various food may also occur while cooking at home or in restaurants. And, finally, we must not forget that the hygienic practices of the consumers themselves affect the probability of acquiring infection.

There are 3 types of hazards, which can be found in food: physical hazards such as glass or other hard foreign objects, chemical hazards which may occur naturally or be added intentionally such as antibiotics, and biological hazards such as microorganism. Humans can be infected with various microorganisms through consumption of contaminated food. These microbial agents include many species of bacteria such as Salmonella, viruses such as hepatitis virus, protozoa such as Cryptosporidium, parasites such as Taenia, and even prions like those causing bovine spongiform encephalopathy and Creutzfeldt-Jakob disease. Bacteria in food may cause disease from the toxins they produce (e.g., Clostridium botulinum, which may sporulate after secretion of toxin into the food). Some bacteria may establish themselves and multiply in the gastrointestinal tract where they cause inflammation. Some of the infected species may invade further into the bloodstream causing septicemia (e.g., Salmonella enterica serovar Typhimurium). Some bacteria such as Shigella spp. may continuously produce toxins which cause severe gastroenteritis. The clinical signs of food borne diseases differ depending on the agent and host characteristic, but usually involve diarrhea, fever and abdominal pain. People affected by biological hazard in food may have to stop working result in loses of income and money through medical care.

In order to mitigate the risk of foodborne disease, we may apply the concept of risk analysis to food safety issues. Risk analysis is a fundamental scientific methodology underlying the development of food safety standard by providing quantitative or qualitative assessment of the risk, method for limiting those risk and ways to create public awareness of the risk and its consequence. Risk analysis consists of 3 components; risk assessment, risk management, and risk communication. Risk assessment is the estimation of the incidence of disease resulting from the hazard. Risk management specifies the intervention necessary to reduce the incidence of disease, while risk communication is the means used to disseminate information and create an understanding of the risk in the population(2).

Risk assessment is also the scientific basis for setting food standards recommended by the World Trade Organization (WTO) (3). It is comprised of 4 steps: hazard identification, exposure assessment, hazard characterization, and risk characterization. In hazard identification, apart from describing the natural history of the hazard, factors affecting growth and survival of the microbial hazard may also be identified. In exposure assessment, the probability of exposure (PE) to the hazard and the dose or amount of hazard in the food is estimated. The hazard characterization steps, which can be called a dose-response assessment (4), involve modeling of the probability of illness (PI) based on the amount of hazard consumed. Finally, risk characterization calculates the probability of disease (PD) in the population from PE and PI. A sensitivity analysis can also be performed in addition to calculation of PD(5). The results of this analysis would indicate the level of influence of each factor on the probability of disease in the population based on a correlation coefficient. Factors with the highest correlation coefficients would be the most effective factors for reducing the risk in the population; in other words, it is an intervention for risk management.

Table 2. Proportion of pathogens isolated from diarrhea patients (%)

	Campylobacter	Salmonella	Rotavirus	Others
Thailand[7]	14.1	3.1	17.2	65.6
Lao PDR[9]	2.9	0.6	21.5	75.0
Vietnam[10]			46.7	53.3

Epidemiology of Salmonella and Campylobacter in Thailand, Laos PDR and Vietnam

The incidence of foodborne disease in Thailand was 248 cases per 100,000 people in 2006, which was much higher than the incidence of avian influenza during the same period of time (0.01 case/100,000)[6]. Although avian influenza may be a more severe disease, foodborne diseases affect many more people. While rotavirus was the most commonly isolated etiologic agent of diarrhea from patients in Thailand[7], Laos PDR[8,9], and Vietnam[10,] *Campylobacter* and *Salmonella* were also found in Thailand and Lao PDR (Table 2).

It has been suggested that the increase of foodborne disease, particularly in industrialized countries, may be the result of changes in the livestock industry as well as changing lifestyles or consumer eating behavior, increased traveling, greater pathogen virulence, changing population demography, and improvements in diagnostic capacity. In Thailand, Lao PDR and Vietnam, *Campylobacter* was the most frequently isolated bacteria in children with foodborne gastrointestinal disease followed by *Salmonella*, *Shigella* and *E.coli*. Although, there are several significant food borne bacteria[11], *Salmonella* and *Campylobacter* has been the focus of attention in veterinary public health field since the main source of these bacteria are food of animal origin. Apart from public health significance, *Salmonella* are also important pathogen for international food trade. Several countries impose zero limit for *Salmonella* contamination in import food[2]. Therefore, we shall focus our attention on these two bacteria.

Salmonella is a gram negative bacterium in the family Enterobacteriaceae, which also include *E.coli*, *Proteus* and several other species. Epidemiologically, we may divide Salmonella into 2 groups: typhoidal *Salmonella* (*S. typhi* and *S. paratyphi*), which only infects humans; and nontyphoidal *Salmonella* (*S. enterica* and *S. bongori*), which infects animals and may be transmitted to humans via food[11]. People are commonly infected with Salmonella through consumption of contaminated food. The incubation period of foodborne salmonellosis ranges from 6 to 48 hours. Its clinical signs include fever, headache, nausea, vomiting, abdominal pain, and diarrhea, which are common among gastrointestinal infections. Some serotypes of *Salmonella* (*S. enterica* serovar Typhimurium or Enterica) may invade the bloodstream and cause septicemia. Although the incidence of foodborne disease in Thailand rose steadily during the past 10 years, the proportion of Salmonella infections per person has been quite unchanged despite some fluctuation[6]. Table 3 showed serotypes of *Salmonella* causing human clinical cases in Thailand. These serotypes were also commonly found in pork and chicken meat.

The significance of *Salmonella* spp. as foodborne pathogens has been documented in many countries including Thailand. Preslaughter pigs and chickens have been incriminated as the major sources of *Salmonella* contaminations of chicken carcasses and pork products at later stages in the food chain[12,13]. Previous studies in Thailand[14] and Vietnam[15] reported low prevalence of Salmonella isolated from preslaughter pigs (5~10%) and chickens (4~8%). Serological testing of pigs yielded much higher seroprevalence (60%)[16,17], indicating that pigs may be exposed to *Salmonella* when they are raised, but may not shed the bacteria at the farm. However, when pork and chicken meat samples were collected from the same area during the same period of time, the prevalence of *Salmonella* contamination is increased in both pork (29~70%) and chicken meat (43~57%)(14). In Thailand, a survey of raw pork preparation showed up to 56% *Salmonella* contamination in Nham (raw pork sausage) and 19% contamination in Laabdib (raw minced meat salad)[18].

Apart from molecular assays, we may use traditional serotyping methods to indicate relationships between *Salmonella* isolated from different sources. During 1990 to 1993, the prevalence of *S. entertidis* in humans increased from 1.3% to 16.98%, while the prevalence of *S. enteritidis* in animals increased from 0.3% to 15.87% in Thailand(19), which suggested the correlation between *Salmonella* in human and animals. The increased prevalence observed may be the result of increasing surveillance or expanding livestock industry. In Thailand, *S. rissen* and *S. Weltevreden* can be commonly found in food animals, meat, and diarrhea patients (Table 3). Another study by WHO on Salmonella and Shigella center in Bangkok also reported that *S. Weltevreden* and *S. enteritidis* are commonly found in humans and frozen food of animal origin[20]. These observations suggested that livestock may be an important source of salmonellosis in Thailand. However, a study in children aged less than 5 years old in Thailand found no significant association between consumption of chicken, pork, or milk with *Salmonella* infection[14], suggesting that there may be other sources of *Salmonella* infection in children.

Since food animals have been implicated as sources of *Salmonella* for human infection, studies have been conducted to determine factors associated with *Salmonella* contamination along the pork production chain. A study in preslaughter pigs in Thailand[16] using *Salmonella* isolation result as outcome showed that pigs with *Salmonella* were more likely to come from medium farm size, having department of livestock development certificate, use effective microorganism as feed additive, having slurry waste management and less number of pigs per pen. No results from the pig slaughterhouses and the markets are available yet.

In the chicken production chain, there were no reports of risk factor studies in chicken farms in this area. A study in Vietnam[21] showed that *Salmonella* contaminated carcasses were more likely to come from intensive farm, processed in small slaughterhouses, use of unchlorinated water and fail to clean work areas frequently

Table 3. Serotypes of Salmonella in food animals, meat and diarrhea patient in Thailand, Lao PDR and Vietnam[20]

	Thailand	Lao	Vietnam
Pig	Rissen, Derby	-	-
Pork	Weltevreden, Rissen, Anatum, Emek	Rissen, Anatum, Derby	Derby, Weltevreden, London
Chicken	Emek, Rissen, Enteritidis	-	-
Chicken meat	Weltevreden, Emek, Hardar	-	Agona, London, Emek
Human	Weltevreden, Rissen, Stanley, Enteritidis, Anatum	-	-

An additional issue of concern in foodborne bacterial infections is antimicrobial resistance. Generally, infection with nonvirulent strains of *Salmonella* may not require antimicrobial treatment. However, antimicrobial treatment may be necessary for immunocompromized patients or those infected with virulent strains. Antimicrobial agents may inhibit growth or kill the bacteria by inhibiting synthesis of cell walls (betalactams), nucleic acids (fluoroquinolones), or proteins (tetracycline and macrolides). Some of these resistance mechanisms may be transferred horizontally among similar species and across species of bacteria via plasmids or integrons[22].

A previous study[23] reported a higher proportion of *Salmonella* isolated in Thailand with resistance to antimicrobial agents used for treatment of gastrointestinal infection, with 28% and 59% of isolates resistant to ampicillin and tetracycline, respectively. Resistance to new agents such as ciprofloxacin (0.5%) and azithromycin (5%) has already been found in Thailand. It has been suggested that antimicrobial agents used in livestock production may contribute to the increase of resistant bacteria, which may subsequently infect humans[24]. A study of standard farms where veterinarians control antimicrobial use and nonstandard farms where farmers administer antimicrobial agents freely showed that *Salmonella* isolated from nonstandard farms were resistant to more types of antimicrobial agents than those isolated from standard farms[14]. However, when we examine the resistance to newer agents such as ciprofloxacin, no resistance was found in food animals, while resistance was common among food animals and humans for traditional drugs such as tetracycline[23]. This observation suggested that resistance mediated by transferable genetic elements was shared between humans and animals, but resistance mediated by mutation of the chromosome (such as resistance to fluoroquinolone, resulting from *gyrA* gene mutation) may be the result of drugs used in humans.

Tetracycline resistance is mediated by a mobile resistance gene usually clustered together in an integron capable of horizontal transfer[25,26]. Detection of the integrase gene in the bacteria indicates the presence of an integron element which may carry an antimicrobial resistance gene. A recent study in Thailand identified this integrase gene in 95% of *Salmonella* isolated from food animals with resistance to oxacillin, erythromycin tetracycline and trimethoprimsulfamethoxazole[27]. Therefore, it is possible that resistance to these agents may result from antimicrobials used in food animals then transferred to humans, or vice versa.

Campylobacter is a gram negative spiral rod bacterium, which grows well in an environment similar to the intestinal tract with 510% CO2, but it is a microaerophile and was not detected for many years because of this growth property. Most *Campylobacter* grow at 37ºC, but some *Campylobacter* also grow at 42ºC, which is the normal body temperature of chickens. *C. jejuni* and *C.coli* are two common species of *Campylobacter* causing foodborne gastroenteritis in humans. These two species of *Campylobacter* do not cause disease in any food animals; therefore, infected food animals may be processed through the food production chain and pose a threat to the consumer undetected. Other species of *Campylobacter* including C. fetus and *C. venerealis* can cause reproductive disease in cattle and pigs[28]. It has been found in chicken meat, unpasteurized milk, untreated water, and in the intestinal tracts of several animals including cats, dogs, poultry, wild birds, swine, cattle, rodents, and monkeys.

The incubation period of Campylobacter infection in human is 1 -11 days, and as few as 500 organisms may be sufficient to cause illness. Clinical signs of Campylobacteriosis are similar to salmonellosis, which include fever, severe abdominal cramps, nausea, vomiting, and diarrhea. Campylobacteriosis is not a common disease in adult in Thailand. Most patients were children age less than 5 years old particularly those immunocompromised children. Campylobacteriosis does not require treatment in adults, but may require antimicrobial treatment in children or immunocompromised patients. *C. jejuni* has also been associated with GuillainBarre syndrome, an autoimmune neurological disease in humans [29].

The prevalence of *Campylobacter* in preslaughter pigs and chickens in Thailand was estimated to be 73% and 64%, respectively, but the prevalence on pork and chicken meat was lower at 23% and 47%, respectively[30]. In Laos PDR, only 3 out of 184 cecum samples from cattle were contaminated with *Campylobacter spp*[31]. In Vietnam the prevalence of Campylobacter in chicken meat was reported to be 35%[21]. The lower prevalence of contamination in meat suggests that pigs and chickens infected at the farm may be processed through the slaughterhouse to the market.

Various molecular techniques may be employed to study the relationship between bacteria isolated from different sources. When a singlestrand conformation polymorphism (SSCP) analysis was applied to *Campylobacter* isolated from food animal and diarrhea patients in Thailand, the results showed that a large proportion of *Campylobacter* share a similar profile, which suggests a clonal spread of *Campylobacter* among humans and food animals[30]. However, whether food animals are important sources of *Campylobacter* infection in humans in Thailand is not completely understood. Padungtod and Kaneene[30] showed that in children less than 5 years old with diarrhea, infected children were more likely to consume chicken than those not infected. When we examine the species of *Campylobacter* in human and food animals, *C. jejuni* was commonly found in dairy cows and humans. While *C. coli* was commonly found in pigs and chickens, but in humans at a far lower proportion. Therefore, dairy products may be more important sources of *Campylobacter* in Thailand than pigs and chickens. Further investigation of this observation should be persue to clarify the factors associated with transmission of *Campylobacter* through milk.

Macrolides (such as erythromycin or azithromycin) and fluoroquinolones (such as ciprofloxacin) are drugs of choice for the treatment of Campylobacteriosis in humans. In Thailand, a high proportion of resistance to ciprofloxacin (77%) was found in *Campylobacter* together with some resistance to azithromycin (6%). On the other hand, a low level of fluoroquinolone resistance (7%) and no azithromycin resistance was found in Vietnam [23]. Since most fluoroquinolone resistance in *C. jejuni* in Thailand was mediated by mutation in the *gyrA* gene[32], which can not transfer horizontally, it is likely that Campylobacter develops resistance in food animals during the raising period on farms, and subsequently infects humans. However, for other antimicrobial agents with mobile resistance genes[33], Campylobacter may develop resistance in food animals and transfer the resistance gene to other human pathogens similar to Salmonella[34].

Summary

Salmonella and *Campylobacter* are important food borne bacteria in Thailand, Lao PDR, and Vietnam. And food animals are major source of these bacteria for human infection. Apart from diseases cased by *Salmonella* and *Campylobacter*, these bacteria may developed and transfer antimicrobial resistance gene to other pathogenic bacteria causing infection, which may not be able to treat with available antimicrobial agents. And we may employ risk analysis framework to evaluate the impact and manage the risk of diseases from these bacteria in Thailand, Lao PDR, and Vietnam.

In order to apply risk analysis framework mentioned earlier, it is essential to obtain information regarding the exposure assessment including the probability and dose of bacteria in various food commodities in the area. It is also necessary to obtain reasonable doseresponse models, which may differ from those in developed countries, for populations in this region. With such information, the probability of illness resulting from hazards in the food can be estimated along with factors affecting the probability. Therefore, surveillance and collaborative research among these three countries is essential to clarifying the epidemiology of foodborne infections common in the region. These results and a thorough understanding of foodhandling techniques at home and in commercial enterprises may also facilitate controlling of the bacteria contamination in food animals and food of animal origin, thus reducing the risk of foodborne diseases in the region and importing countries.

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