Epidemiological dynamic potential of transboundary *Salmonella* spp. in meat sold at retail markets in the middle Mekong basin area (#53241)

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Epidemiological dynamic potential of transboundary *Salmonella* spp. in meat sold at retail markets in the middle Mekong basin area

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Background: The surrounding area of the middle Mekong basin, particularly along the border line between Thailand and Lao People's Democratic Republic (Lao PDR), is considered to be a high-risk area for many livestock-associated foodborne illnesses, especially salmonellosis. The aim of this study was to determine the prevalence and characteristics of *Salmonella* spp. contaminated in pork, beef and chicken meats sold at retail markets in the Thailand-Laos border area surrounding the Thai-Lao Friendship Bridge I from January to May 2019. This study focused on the characteristics of the serotype, antimicrobial susceptibility testing and the genotype based on multilocus sequence typing (MLST) of the relevant *Salmonella* strains. The findings will make a significant contribution to the current understanding of *Salmonella* epidemiology with the aim of enhancing food security in the region. **Results:** From a total of 370 meat samples collected, 63% were found to be positive for *Salmonella*, with the prevalence of 73%, 60% and 56% from pork, beef and chicken meat samples, respectively. Of all positive samples, 53 serotypes were identified. Of these, *S*. London accounted for the majority (27%), followed by *S*. Corvallis (14%), and *S*. Rissen (6%). Resistance against tetracycline was found at the highest frequency (50%), followed by ampicillin (35%) and sulfamethoxazole-trimethoprim (28%). MLST revealed that no evidence of shared genetical relatedness of *Salmonella* at retail's sites among Thailand-Laos border zone. However, a diverse range of their genotypes had been spread over the area. Besides, persistence of the residential pathogen and sharing of the supply route within-country can be inferred. **Conclusions:** Regular disinfecting of all working areas along with quality control checking at the pre-retail stage must be applied to reduce the transmission of *Salmonella* as well other foodborne pathogens downstream consumers.

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Abstract

Background: The surrounding area of the middle Mekong basin, particularly along the border line between Thailand and Lao People's Democratic Republic (Lao PDR), is considered to be a high-risk area for many livestock-associated foodborne illnesses, especially salmonellosis. The aim of this study was to determine the prevalence and characteristics of *Salmonella* spp. contaminated in pork, beef and chicken meats sold at retail markets in the Thailand-Laos border area surrounding the Thai-Lao Friendship Bridge I from January to May 2019. This study focused on the characteristics of the serotype, antimicrobial susceptibility testing and the genotype based on multilocus sequence typing (MLST) of the relevant *Salmonella* strains. The findings will make a significant contribution to the current understanding of *Salmonella* epidemiology with the aim of enhancing food security in the region.

Results: From a total of 370 meat samples collected, 63% were found to be positive for *Salmonella*, with the prevalence of 73%, 60% and 56% from pork, beef and chicken meat samples, respectively. Of all positive samples, 53 serotypes were identified. Of these, *S*. London accounted for the majority (27%), followed by *S*. Corvallis (14%), and *S*. Rissen (6%). Resistance against tetracycline was found at the highest frequency (50%), followed by ampicillin (35%) and sulfamethoxazole-trimethoprim (28%). MLST revealed that no evidence of shared genetical relatedness of *Salmonella* at retail's sites among Thailand-Laos border zone. However, a diverse range of their genotypes had been spread over the area. Besides, persistence of the residential pathogen and sharing of the supply route within-country can be inferred.

Conclusions: Regular disinfecting of all working areas along with quality control checking at the pre-retail stage must be applied to reduce the transmission of *Salmonella* as well other foodborne pathogens downstream consumers.

Key words: *Salmonella*; retail market; MLST; Thailand-Laos border**;** middle Mekong basin

Introduction

Salmonella spp. is an important causative agent of certain foodborne illnesses in humans and can be found all over the world (Sirichote et al., 2010; Van Boxstael et al., 2012; Basler et al., 2016). Annually, ninety million cases resulting in 150,000 deaths among salmonellosis patients have been counted (Campioni et al., 2012). Along with the direct effects of *Salmonella* spp. on the gastrointestinal tract, the evidence of drug resistance is also a major public health concern (Foley & Lynne, 2008; Kurtz et al., 2017; Jajere, 2019). Consequently, a reduction in the first line of empirical treatments and limitations associated with treatment choices are listed as the main factors that have influenced the current situation (Van Boxstael et al., 2012).

Livestock products (farm animal-origin food), especially meats, are known to be an important source of human salmonellosis (Heyndrickx et al., 2002; Mainali et al., 2009; Rostagno & Callaway, 2012). Considering the food production chain, retail markets have been identified as the most significant point of contact for salmonellosis exposure and transmission among humans (Hauser et al., 2011; Gomes-Neves et al., 2012). Furthermore, improper management at locations ahead of the marketplace, such as farms and/or slaughterhouses, also contribute to the chances of increased pathogen loads at the retail site. This can occur either directly or through contaminated equipment due to improper handling practices or unsuitable storage conditions (Lo Fo Wong et al., 2002).

Nowadays, an increase in demand has led to an intensive transformation of the animal production industry (Guardabassi et al., 2008). Good Management Practices (GMP) must be implemented at all levels of production to ensure an optimum level of food safety for consumers. However, these practices cannot be 100% implemented in certain developing regions such as the middle Mekong basin and the surrounding area, particularly along the border between Thailand and Lao People's Democratic Republic (Lao PDR). Animal farming practices are being used extensively in traditional free-range and small holder backyard systems, which often employ the minimum in terms of hygienic sanitation systems. Moreover, inadequate practices in slaughterhouses and retail outlets, such as on-floor slaughtering and uncontrolled storage conditions in purchasing areas, are considered incautious methods of practice. Additionally, cultural preferences and an inadequate level of awareness among local people with regard to raw meat consumption, as well as an absence of reliable and high-quality resources, such as clean water and cooking supplies, are also considered important risk factors (Wilson, 2007; Conlan et al., 2014; Okello et al., 2017). All of the above-mentioned situational conditions are acknowledged as the potential predisposing factors of several foodborne diseases such as salmonellosis (Mughini-Gras et al., 2014; Ferrari et al., 2019). In 1994, the Thai-Lao Friendship Bridge I linking Nong Khai Province, Thailand and the Vientiane Capital of Lao PDR was officially opened. More than three million people cross the bridge each year (Australian Embassy Thailand, 2011). These people cross by way of both private and public vehicles to engage in trade and the exchange of various products. Therefore, the chances of pathogen transmissions between border line communities are likely to be high.

In a study of *Salmonella* prevalence in meat being sold surrounding the Thailand-Laos border region, Sinwat et al., (2016) reported that the prevalence of pork contamination in the border areas of Thailand and Laos PDR was 65%. Moreover, Boonmar et al., (2013) reported that the prevalence of contaminated pork and beef in southern-Laos PDR was 93% and 82%, respectively. Both studies reported a widespread variety of serotype distribution including *S*. Typhimurium, *S*. Derby, *S*. Anatum or *S*. Rissen. Interestingly, almost all strains of *Salmonella* are currently classified as multidrug-resistant strains. However, based on the data that has been studied, there is a clear lack of reported information on poultry meat, while it is well known that poultry meat is commonly consumed in this area. Additionally, in order to gain access to in-depth epidemiological information, a study of the pathogen characteristics at the genetic level should be fulfilled in order to expand upon the scope of epidemiological knowledge of these pathogens. Multilocus sequence typing (MLST) technique is one of the methods employed in our study. This technique relies on a comparison of the sequences of allele types in a specific group for each set of house-keeping gene and focuses on the genetics in order to assemble data of the sequence type (ST). The resulting findings can be compared with the information presented in a number of databases related to the study of global bacterial dynamic distribution and their genetic evolution (Liu et al., 2011; Patchanee et al., 2017).

The purpose of the study was to investigate the prevalence of *Salmonella* in meat sold at retail markets in the middle Mekong Basin area along the border of Thailand and Lao PDR, surrounding the Thai-Lao Friendship Bridge I. Together with comparing the expressive and characteristics (serotypes, antimicrobial resistant patterns and ST type) of *Salmonella* to expand upon the epidemiological knowledge of pathogen distribution. The findings from this study would be extremely useful in the creation of appropriate preventive measures that would help control the spread of human salmonellosis in the region.

Materials and methods

Sample collection

The sample size of this study was determined using the Win Epi online program (http://www.winepi.net/uk/index.htm). For each parameter used, the degree of prevalence identified in the previous study was used as the "expected prevalence". Rates of 65% (Sinwat et al., 2016), 73% (Trongjit et al., 2017) and 82% (Boonmar et al., 2013) were chosen to calculate the sample sizes for pork, chicken and beef, respectively. Furthermore, 8.5% and 95% were selected as the "accepted error" and "confidence level" values, respectively for the required feature inputs. For the infinite population, a minimum of 121, 105 and 79 samples of pork, chicken and beef were designated, respectively. However, in order to achieve even greater levels of accuracy and reliability, additional samples were carefully chosen.

During the period of time from January to May, 2019, 370 samples (121, 133 and 116 samples of pork, chicken and beef, respectively) were carefully collected from five retail markets in Vientiane Capital, Lao PDR and six retail markets in Nong Khai Province, Thailand. All samples were then individually labeled, put into plastic packs and stored in an icebox for laboratory analysis within 24 hr at the Department of Veterinary Medicine, Faculty of Agriculture, National University of Laos, Nabong Campus, Lao PDR.

Salmonella isolation and identification

Isolation and identification of *Salmonella* spp. from meat samples (pork, chicken, beef) were performed following ISO6579: 2002 Amendment 1:2007, Annex D technique. Accordingly, 25 g of samples were enriched with 225 ml of Buffered Peptone Water (BPW; Merck, Germany). The mixing materials were homogenized for 120 sec and incubated at 37ºC for 18-24 hr. An aliquot of 100 µl of the pre-enriched inoculum was transferred to 10 ml of Rappaport-Vassiliadis broth (RV; Oxoid, England) at 42ºC for an incubation period of 24-48 hr. The likely colonies were streaked on Xylose-Lysine-Desocholate agar (XLD; Oxoid, UK) and Brilliant-green Phenol Red Lactose sucrose agar (BPLS; Merck, Germany). After an incubation period of 18-24 hr at 37ºC, the plates were then inspected. The presumptive colonies were then placed on Triple Sugar Iron agar (TSI; Oxoid, England), urease and Motility Indole-Lysine agar (MIL; Merck, Germany). An analysis of the strains indicated a correct bio-chemical reaction as positive for *Salmonella*. Finally, all results were then recorded. *Salmonella* prevalence with a 95% confidence level along with evidence of an association between a *Salmonella* positive proportion and the relevant specifications (location or meat type) were determined by descriptive statistical analysis and fisher's exact test, respectively. All analyses were completed using Epi Info^{TM} version 7. Statistically significant levels were determined at $p<0.05$.

Serotyping and Antimicrobial susceptibility testing

All detected *Salmonella* spp. specimens were serotyped using the serum-agglutination test according to the White-Kauffmann-Le Minor scheme (Popoff et al., 1993). This was made possible by a contribution from the Bacteriology Section, Veterinary Research and Development Center (Upper Northern Region), Lampang, Thailand. All strains were also run through antimicrobial susceptibility testing with agar disk diffusion using ten panels of antimicrobial agents. These agents were comprised of amoxicillin-clavulanic acid (AMC) 20/10 µg, ampicillin (AMP) 10 µg, chloramphenicol (C) 30 µg, ciprofloxacin (CIP) 5 µg, cefotaxime (CTX) 30 µg, nalidixic acid (NA) 30 µg, norfloxacin (NOR) 10 µg, streptomycin (S) 10 µg, sulfamethoxazole-trimethoprim (SXT) 23.75/1.25 µg and tetracycline (TE) 30 µg. (CLSI, 2011). Strains that existed with intermediate resistance were grouped as being susceptible in order to avoid overestimation. Strains that resisted ≥ 3 of the antimicrobial agents were considered multidrug resistant.

Multilocus sequence typing (MLST)

Randomly selected specimens of 38 *Salmonella* obtained from the most frequently found serotypes were genotyped using the MLST technique. Total numbers of DNA genomics were extracted according the protocol described by Liu et al., (2011). Seven housekeeping genes, including *aro*C (chorismate synthase); *dna*N (DNA polymerase III beta subunit); *hem*D (uroporphyrinogenIII cosynthase); *pur*E (phosphoribosylaminoimidazole carboxylase); *suc*A (alpha ketoglutarate dehydrogenase); *his*D (histidinol dehydrogenase) and *thr*A (aspartokinase I/homoserine dehydrogenase), were selected for MLST profiling. PCR amplification of all 7 genes was accomplished using the method previously described by Kotetishvil et al., (2002). Subsequently, the products were sent to be sequenced at the Macrogen Service Center, Republic of Korea.

The sequences obtained in each gene (**Supplementary file 1**) were transformed into allele numbers, and were compiled according to the sequence type (ST) data obtained from the http://mlst.warwick.ac.uk/mlst/ database. Finally, all data of the *Salmonella* strains acquired from this study were analysed. A phylogenetic tree was constructed using Bionumerics® software version 7.6 (Applied Maths, Belgium).

Results

A total of 370 meat samples were collected during the period of January to May 2019. Subsequently, 135 samples from six retail markets in Nong Khai Province, Thailand and 235 samples from five in Vientiane Capital, Lao PDR were included. With regard to the meat types, 121, 133 and 116 samples of pork, chicken and beef were obtained, respectively (**Table S1**). The overall prevalence of *Salmonella* spp. was found to be 62.70% (232/370; 95% CI: 57.67-67.48%). In consideration of the sampling locations, the degree of prevalence of the samples collected from Laos PDR (70.21%; 165/235) was statistically higher than the samples collected from the Thai sampling sites (49.63%; 67/135) ($p<0.05$). In terms of measuring each meat type, the degree of prevalence of the *Salmonella* spp. positive results found in pork, chicken and beef were 72.73% (88/121), 55.64% (74/133) and 60.34% (70/116) in that order. The percentage of prevalence from the samples recovered from pork was significantly higher than from the chicken samples ($p<0.05$), but this figure tended to be higher comparing with the beef samples ($p=0.05$). **Table 1** demonstrates the distribution details of *Salmonella* spp. positives with 95% confidence intervals among the different locations and sample types.

From the overall *Salmonella* strains, 53 serotypes were identified by serum-agglutination according to the White-Kauffmann-Le Minor scheme (**Table 2**). The highest degree of frequency was found in *S*. London (26.99%; 61/232), followed by *S*. Corvallis (13.79%; 32/232), *S*. Rissen (6.47%; 15/232) and *S*. Weltevreden (6.03%; 14/232), respectively. With regard to the locations of the collected samples, those 4 serotypes were also noted as being in the majority for all locations with the exception of *S*. London, only 2 strains were identified in the Thai samples. Stratifying for each meat type, *S*. London was the most common serotype detected from the pork and beef samples. For the chicken samples, the highest degree of frequency was found for *S*. Corvallis. Interestingly, there were just three serotypes, *S*. London, *S*. Rissen, *S*. Corvallis and *S*. Typhimurium, that were distributed among all meat types.

All 232 *Salmonella* strains were submitted for antimicrobial susceptibility testing. Consequently, 76 of them (32.75%) were classified as multidrug resistant strains. Resistance to tetracycline (49.57%; 115/232) was found in the highest frequency, followed by ampicillin (35.34%; 82/232), and sulfamethoxazole-trimethoprim (28.45%; 66/232), respectively. On the other hand, 94 strains (40.52%) were found to be susceptible to all of the tested antimicrobials. Additionally, only 3 strains (1.29%) were found to be resistant to cefotaxime, while 5 (2.16%) and 6 strains (2.59%) were found to be resistant to norfloxacin and ciprofloxacin, respectively. In terms of the locations from which the samples were obtained, tetracycline (57.78%; 95/165), ampicillin (34.55%; 57/165) and sulfamethoxazole-trimethoprim (32.12%; 53/165) were also ranked in the top most three antimicrobials demonstrating a high resistant rate in Laos PDR. Then again, in Thailand, the resistant rates were ranked as follows; ampicillin (37.31%; 25/67), tetracycline (29.85%; 20/67) and streptomycin (20.90%; 14/67), respectively (**Fig. 1**). With regard to meat type, resistance rates were divided for each group and are also displayed in **Fig. 1**. Generally, those rates were found to be somewhat different. Interestingly, especially in the effective antimicrobials, strains resisted against norfloxacin or ciprofloxacin were almost originated from chicken meat. Thus related with the individual data, there were three strain resisted at least seven antimicrobials test (AMP / AMC / CIP / NA / NOR / SXT / TE, AMP / AMC / C / CIP / NA /

NOR / SXT / TE and AMP / AMC / C / CIP / NA / NOR / S / SXT / TE), all three were isolated from the chicken meat from Nong Khai, Thailand.

Fig. 2 demonstrates the genetic relatedness of *Salmonella* currently being detected at the Thailand-Laos border area. From the 38 *Salmonella* strains analysed, 35 genetic characters were found. The 28 genotypes could not be identified to any ST, and 11 of them could not be grouped in any ST-complex. One to five variation of housekeeping genes of those 28 un-identifies from known ST were displayed in **Table 3**. However, for the 7 nameable STs (ST64, ST155, ST365, ST469, ST516, ST1541, ST5706), four of those were distinct to a single strain. Nonetheless, two strains were grouped in the ST155, ST365 and ST 469. Strains grouped in ST155 were derived from a Lao-Aussi market, one was chicken meats and the another was pork. Two strains of ST365 originated from Phonsavang market, one was chicken and the another was beef were also identified. Additionally, the strains grouped in ST469 were originated from pork and beef samples collected from different markets located in nearby areas.

Discussion

The findings obtained from this study represent scientific information on the burden and intensity of *Salmonella* in livestock meats in a 30 km radius surrounding the Thai-Lao Friendship Bridge I. It is well known that this bridge is the primary place for the expansion of trade that has occurred along the Thailand and Laos PDR border since 1994.

Overall degree of prevalence of *Salmonella* spp. in all meat samples was found to be 63%. With regard to each meat type, pork, chicken and beef were found to be contaminated with pathogens at rates of 73%, 56% and 60%, respectively. The degree of contamination in pork had a tendency to be higher in all collected samples in a study conducted by Sinwat et al., (2016). The degree of intensity was recorded at 65.06% (95% CI: 59.26-70.47%). However, for beef samples, the degree of prevalence in this study was lower when compared to the results of a study conducted by Boonmar et al., (2013), in which the prevalence was recorded at 82.35% (95% CI: 58.97-93.81%). Actually, comprehensive figures related to *Salmonella* contamination in the chicken meat being sold in the border area of Thailand and Laos have not yet been reported. A study conducted by Trongjit et al., (2017) reported on the degree of prevalence of *Salmonella* infection at the Thai-Cambodia border, which was found to be 73.43% (95% CI: 66.65-79.42%). This figure is quite a bit higher when compared with our results. In general, most studies have not demonstrated much difference when reporting numbers, which has been proven by an overlap in the 95% confidence intervals. Variations in the results might depend upon the time period of the sampling along with any existing geographical factors. Nevertheless, high levels of *Salmonella* contamination still persist, even though some intervention has been implemented in some of these areas. *Salmonella* remains a problem in the meat being sold in this region, which has been an important public health concern for the last half decade.

The prevalence of *Salmonella* in the Laos PDR-originated samples was statistically and significantly high. Normally, sanitation practices are different at each location. In fact, there is no supermarket in Laos DPR. All fresh food, such as meat, can only be bought from fresh markets and mini-grocery stores. The lack of covering materials, unsuitable storage conditions and inadequate disinfection practices at the purchasing areas during the meat cutting and handling processes can substantially increase the risk of bacterial colonization (Gomes-Neves et al., 2012; Patchanee et al., 2016). In Thailand, supermarkets are the preferred place to purchase meat. One-sixth of the Thai samples collected in our study were obtained from supermarkets. It is known for stringently regulating their facilities, as well as for implementing biosecurity and hygienic policies along with quality control practices at the pre-harvesting and harvesting stages along the supply chain. However, in general, standard protocols for every type of retail outlet (supermarkets, mini-grocery stores and fresh markets) tend to be higher in bigger cities (Trongjit et al., 2017). With a focus on meat type, pork was found to be the most prevalent when compared with the other types of meat. The reason for this may not be clear. It could be due to the high bacterial loads that emerge during previous production stages, which can then lead to instances of contamination at retail outlets. According to the information obtained from previous studies, *Salmonella* prevalence at pig farms and during pig slaughtering processes was higher than in chicken and beef production (Padungtod & Kaneene, 2006; Trongjit et al., 2017; Phongaran et al., 2019). Another possible explanation for this is that pork is the most common type of meat consumed in this region of study (Napasirth & Napasirth, 2018). The amount of dressing required, along with any other forms of manipulation that are required before meat is sold, would likely increase the opportunity for contamination or re-contamination by product exposure at the final.

Based on the findings of previous studies, *S*. Typhimurium and *S*. Rissen are the majority serotypes identified in pork (Patchanee et al., 2016; Sinwat et al., 2016). For chicken meat, *S*. Corvallis and *S*. Enteritidis are known to be the dominant serotypes (Trongjit et al., 2017). Additionally, *S*. Stanley and *S*. Typhimurium have been reported as the most commonly recorded serotypes in beef (Boonmar et al., 2013). At the moment, these serotypes have not been universally matched to each meat type, but low frequencies were recorded in some instances. However, *S*. Corvallis is still noted as being the dominant serotype in chicken meat. Consequently, new typical sero-characteristics for this region should be set for pork and beef. As the data indicates, *S*. London is presently the most common serotype. Furthermore, as several serotypes have been detected, some of them, such as *S*. Altona, *S*. Cerro, *S*. Elisabethville, *S*. Itami, *S*. Mikamasima, *S*. Ruzizi, etc., have been reported for the very first-time with regard to the isolation of *Salmonella* in the region. Time factor and sample picking, as well as the cross contamination that occurs from other sources, are notable factors. Furthermore, even though the same serotype might be present at several meat origins, it cannot be concluded that two or three meat types would be representative of a sharing pool for *Salmonella* identified from similar sources (Sinwat et al., 2016). All of which would need to be proven later.

Salmonella specimens isolated in this study displayed a relatively high degree of frequency of resistance against tetracycline and ampicillin, which is consistent with the findings of previous investigations (Padungtod & Kaneene, 2006; Pulsrikarn et al., 2012; Boonmar et al., 2013). From the past until now, the antimicrobials have been widely used for treatment and prophylaxis in livestock. However, excessive or inappropriate use is considered to be a key factor that has led to the current situation of resistance (Jajere, 2019). On the contrary, low resistance rates have been recorded for cefotaxime, norfloxacin and ciprofloxacin. Instances of resistance should be of particular concern in resource-limited countries because these are the drugs of choice that are recommended to treat human salmonellosis. Particularly for quinolones (norfloxacin and ciprofloxacin), the antimicrobial class is now often used as the first line of treatment (Kurtz et al., 2017). Of course, all of the quinolone-resistant strains discovered in these study areas originated from chicken meat collected in Thailand, and all were found to be resistant to 7-9 of the tested antimicrobials. Selective pressures such as those associated with antimicrobial use, unsuitable temperatures or pH levels, could be considered sub-lethal stress factors during the short production cycle in broiler farms. Additionally, the chicken's gut ecology should be taken into account when developing resistance to bacterial pathogens (Shang et al., 2018; Yang et al., 2019).

An overview of the phylogenetic tree reveals that most of the strains obtained in this study were diverse. Five-sixths of all strains could not be named to any ST. Microevolution or mutation is possible explanation for the finding (Harbottle et al., 2006; Liu et al., 2011). This may be proven if some unidentified strains differed in only one or two loci from the other known nomenclatures. Moreover, the variation of 3-5 housekeeping genes of known related ST were also denoted. Therefore, of those, many strains cannot be grouped in any clonal complexes. Those that were unidentified may be newly detected STs that have not yet been recorded in the MLST database. At this moment, shared genetical relatedness of *Salmonella* among Thai-Laos border area was not evidenced. The finding infers that the transboundary food supply chain from locations ahead of the marketplace (farm and/or slaughterhouse) are not involve to contamination occurs. However, since only 38 strains were analysed, it cannot 100% prove that *Salmonella* contamination from both countries are not related. An analysis together with information of all geographical matching strains previously submitted in MLST database should be recommended fulfilled the gap, further. Interestingly, various specimens of the *Salmonella* genotypes collected from a single market were remarked. In details, 9 and 6 *Salmonella* genotypes were detected in various meat types collected from Phonsavang and Chengsavang markets, respectively. These findings evidence that more than one infection source can exist at a given location. Contamination can occur by itself in the purchasing area or as a result of inadequate processing when the meat product may have already been contaminated. Unhygienic practices at previous production sites, such as on farms and at slaughterhouses, or at transportation hubs may be causes of transmission in the present situation (Heyndrickx et al., 2002; Campioni et al., 2012).

According to the findings of the ST155 and ST365 clonal strains, both were detected on different sampling dates from the same marketplaces. Persistence of residential *Salmonella* flora could therefore be inferred. Moreover, two strains of ST469 were recovered from different markets at nearby areas. Consequently, the sharing of supply routes at the nearby areas could play a significant role in the dissemination of *Salmonella* spp.

Conclusions

As has been demonstrated, the presence of *Salmonella* in meat sold in the middle Mekong basin area was relatively high in terms of prevalence. Standard hygienic protocols are known to be maintained at a higher level in administrative areas. The findings from the molecular MLST indicate that extensive quality control checking at pre-retail stages should be implemented. Furthermore, regular disinfecting of all equipment, as well as at working areas, must be applied. Future efforts in the strengthening of food safety education and awareness programs would help authorities to establish strategies that could potentially reduce the transmission of *Salmonella* and other foodborne pathogens to downstream consumers.

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Disclosure Statement

No competing financial interests exist.

Figure Legends

Figure 1: Percent resistant against antimicrobials of *Salmonella* isolated from various meat types in the Thai-Lao border area.

Antibiotic abbreviations:amoxicillin-clavulanic acid (AMC); ampicillin (AMP); chloramphenicol (C); ciprofloxacin (CIP); cefotaxime (CTX); nalidixic acid (NA); norfloxacin (NOR); sulfamethoxazole-Trimethoprim (SXT); streptomycin (S); tetracycline (TE).

Figure 2: Dendrogram generated using UPGMA algorithms based on MLST profiles with their phenotypic characterizations and the epidemiological data of *Salmonella* circulating in the Thai-Laos border area.

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Figure 1

Percent resistant against antimicrobials of *Salmonella* isolated from various meat types in the Thai-Lao border area.

Antibiotic abbreviations:amoxicillin-clavulanic acid (AMC); ampicillin (AMP); chloramphenicol (C); ciprofloxacin (CIP); cefotaxime (CTX); nalidixic acid (NA); norfloxacin (NOR); sulfamethoxazole-Trimethoprim (SXT); streptomycin (S); tetracycline (TE).

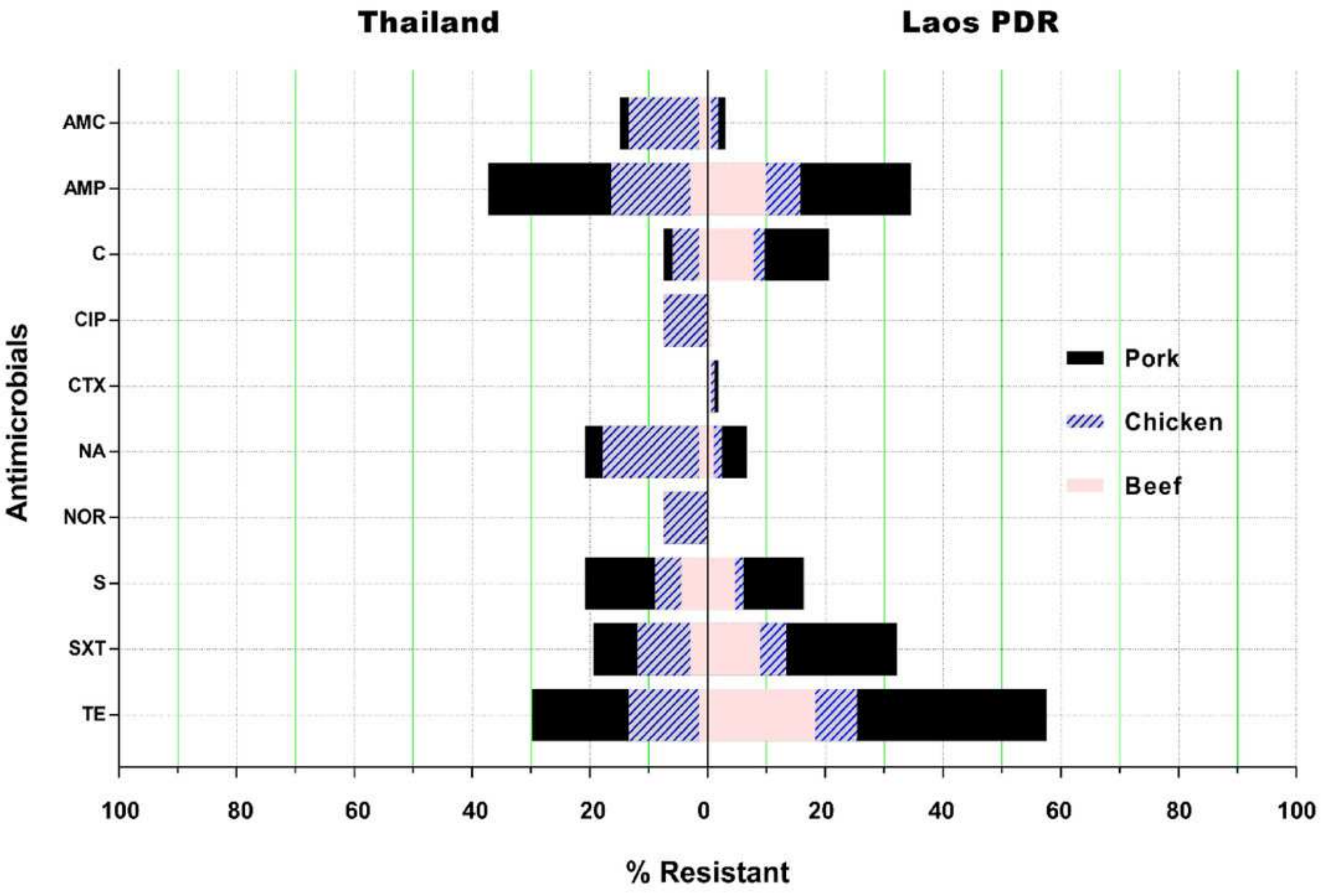
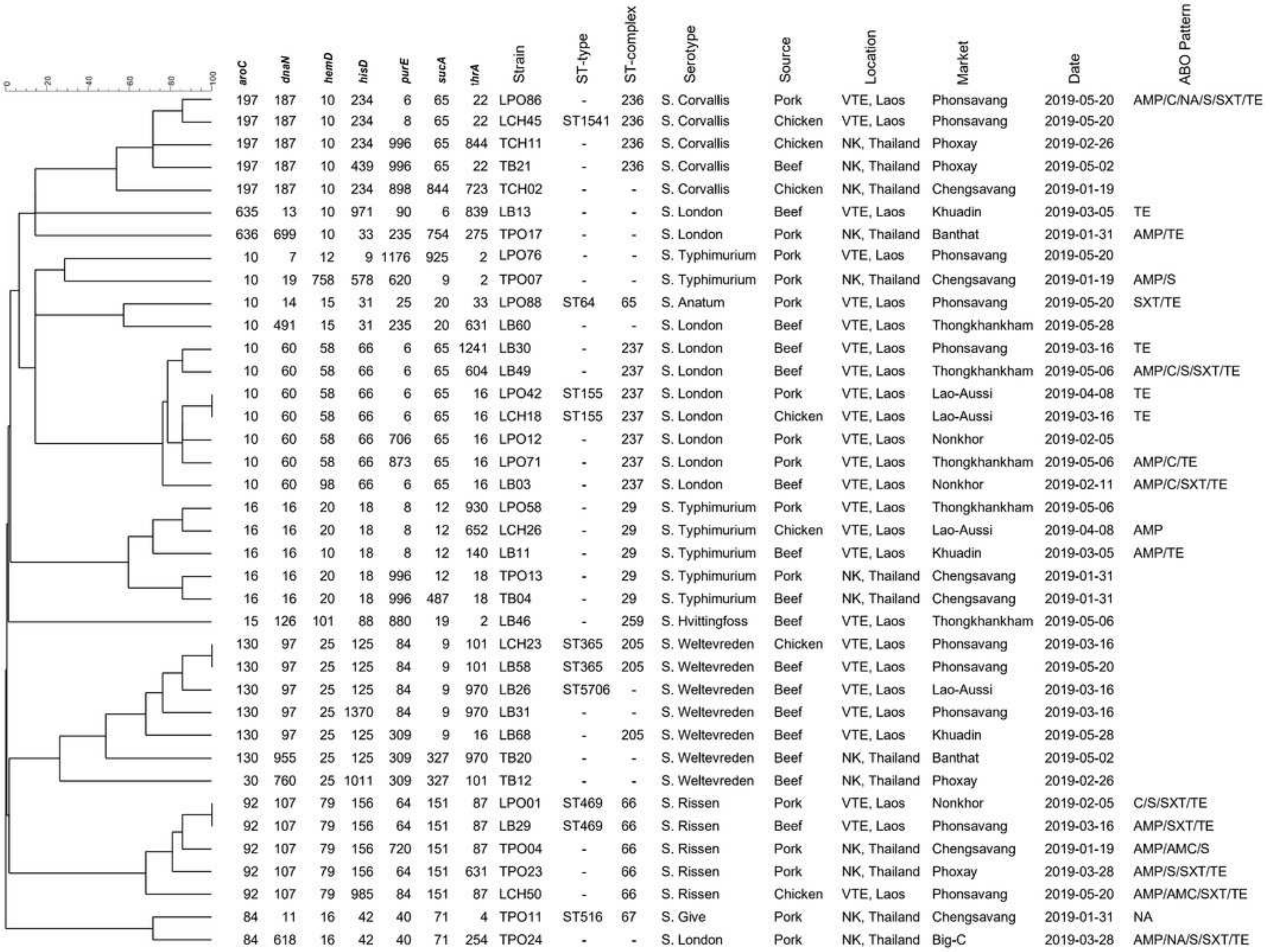


Figure 2

Dendrogram generated using UPGMA algorithms based on MLST profiles with their phenotypic characterizations and the epidemiological data of *Salmonella* circulating in the Thai-Laos border area.



aroC	*dnaN*	*hemD*	*hisD*	*purE*	*sucA*	*thrA*	Strain	ST-type	ST-complex	Serotype	Source	Location	Market	Date	ABO Pattern
197	187	10	234	6	65	22	LPO86	-	236	S. Corvallis	Pork	VTE, Laos	Phonsavang	2019-05-20	AMP/C/NA/S/SXT/TE
197	187	10	234	8	65	22	LCH45	ST1541	236	S. Corvallis	Chicken	VTE, Laos	Phonsavang	2019-05-20	
197	187	10	234	996	65	844	TCH11	-	236	S. Corvallis	Chicken	NK, Thailand	Phoxay	2019-02-26	
197	187	10	439	996	65	22	TB21	-	236	S. Corvallis	Beef	NK, Thailand	Phoxay	2019-05-02	
197	187	10	234	898	844	723	TCH02	-	-	S. Corvallis	Chicken	NK, Thailand	Chengsavang	2019-01-19	
635	13	10	971	90	6	839	LB13	-	-	S. London	Beef	VTE, Laos	Khuadin	2019-03-05	TE
636	699	10	33	235	754	275	TPO17	-	-	S. London	Pork	NK, Thailand	Banthat	2019-01-31	AMP/TE
10	7	12	9	1176	925	2	LPO76	-	-	S. Typhimurium	Pork	VTE, Laos	Phonsavang	2019-05-20	
10	19	758	578	620	9	2	TPO07	-	-	S. Typhimurium	Pork	NK, Thailand	Chengsavang	2019-01-19	AMP/S
10	14	15	31	25	20	33	LPO88	ST64	65	S. Anatum	Pork	VTE, Laos	Phonsavang	2019-05-20	SXT/TE
10	491	15	31	235	20	631	LB60	-	-	S. London	Beef	VTE, Laos	Thongkhankham	2019-05-28	
10	60	58	66	6	65	1241	LB30	-	237	S. London	Beef	VTE, Laos	Phonsavang	2019-03-16	TE
10	60	58	66	6	65	604	LB49	-	237	S. London	Beef	VTE, Laos	Thongkhankham	2019-05-06	AMP/C/S/SXT/TE
10	60	58	66	6	65	16	LPO42	ST155	237	S. London	Pork	VTE, Laos	Lao-Aussi	2019-04-08	TE
10	60	58	66	6	65	16	LCH18	ST155	237	S. London	Chicken	VTE, Laos	Lao-Aussi	2019-03-16	TE
10	60	58	66	706	65	16	LPO12	-	237	S. London	Pork	VTE, Laos	Nonkhor	2019-02-05	
10	60	58	66	873	65	16	LPO71	-	237	S. London	Pork	VTE, Laos	Thongkhankham	2019-05-06	AMP/C/TE
10	60	98	66	6	65	16	LB03	-	237	S. London	Beef	VTE, Laos	Nonkhor	2019-02-11	AMP/C/SXT/TE
16	16	20	18	8	12	930	LPO58	-	29	S. Typhimurium	Pork	VTE, Laos	Thongkhankham	2019-05-06	
16	16	20	18	8	12	652	LCH26	-	29	S. Typhimurium	Chicken	VTE, Laos	Lao-Aussi	2019-04-08	AMP
16	16	10	18	8	12	140	LB11	-	29	S. Typhimurium	Beef	VTE, Laos	Khuadin	2019-03-05	AMP/TE
16	16	20	18	996	12	18	TPO13	-	29	S. Typhimurium	Pork	NK, Thailand	Chengsavang	2019-01-31	
16	16	20	18	996	487	18	TB04	-	29	S. Typhimurium	Beef	NK, Thailand	Chengsavang	2019-01-31	
15	126	101	88	880	19	2	LB46	-	259	S. Hvittingfoss	Beef	VTE, Laos	Thongkhankham	2019-05-06	
130	97	25	125	84	9	101	LCH23	ST365	205	S. Weltevreden	Chicken	VTE, Laos	Phonsavang	2019-03-16	
130	97	25	125	84	9	101	LB58	ST365	205	S. Weltevreden	Beef	VTE, Laos	Phonsavang	2019-05-20	
130	97	25	125	84	9	970	LB26	ST5706	-	S. Weltevreden	Beef	VTE, Laos	Lao-Aussi	2019-03-16	
130	97	25	1370	84	9	970	LB31	-	-	S. Weltevreden	Beef	VTE, Laos	Phonsavang	2019-03-16	
130	97	25	125	309	9	16	LB68	-	205	S. Weltevreden	Beef	VTE, Laos	Khuadin	2019-05-28	
130	955	25	125	309	327	970	TB20	-	-	S. Weltevreden	Beef	NK, Thailand	Banthat	2019-05-02	
30	760	25	1011	309	327	101	TB12	-	-	S. Weltevreden	Beef	NK, Thailand	Phoxay	2019-02-26	
92	107	79	156	64	151	87	LPO01	ST469	66	S. Rissen	Pork	VTE, Laos	Nonkhor	2019-02-05	C/S/SXT/TE
92	107	79	156	64	151	87	LB29	ST469	66	S. Rissen	Beef	VTE, Laos	Phonsavang	2019-03-16	AMP/SXT/TE
92	107	79	156	720	151	87	TPO04	-	66	S. Rissen	Pork	NK, Thailand	Chengsavang	2019-01-19	AMP/AMC/S
92	107	79	156	64	151	631	TPO23	-	66	S. Rissen	Pork	NK, Thailand	Phoxay	2019-03-28	AMP/S/SXT/TE
92	107	79	985	84	151	87	LCH50	-	66	S. Rissen	Chicken	VTE, Laos	Phonsavang	2019-05-20	AMP/AMC/SXT/TE
84	11	16	42	40	71	4	TPO11	ST516	67	S. Give	Pork	NK, Thailand	Chengsavang	2019-01-31	NA
84	618	16	42	40	71	254	TPO24	-	-	S. London	Pork	NK, Thailand	Big-C	2019-03-28	AMP/NA/S/SXT/TE

Table 1(on next page)

Distribution of prevalence and a 95% confidence interval of *Salmonella* isolated from various meat types in the Thai-Lao border area.

Table 1 Distribution of prevalence and a 95% confidence interval of *Salmonella* isolated from various meat types in the Thai-Lao border area.

Type	Location		TOTAL
	Thailand	Laos PDR	
Pork	18/28 (64.29; 44.07-81.36%)	70/93 (75.27; 65.24-83.63%)	88/121^{A} (72.73; 63.88-80.43%)
Chicken	31/66 (46.97; 34.56-59.66%)	43/67 (64.18; 51.53-75.53%)	74/133^{B} (55.64; 46.78-64.25%)
Beef	18/41 (43.90; 28.47-60.25%)	52/75 (69.33; 57.62-79.47%)	70/116^{AB} (60.34; 50.84-69.31%)
TOTAL	67/135^{a} (49.63; 40.92-58.36%)	165/235^{b} (70.21; 63.92-75.98%)	232/370 (62.70; 57.67-67.48%)

Use of fisher's exact analysis and difference of superscript (A, B) indicate significant differences ($p<0.05$) of prevalence detected among meat types. Difference of superscript (a, b) indicates significant differences ($p<0.05$) of prevalence detected among all locations.

Table 2(on next page)

Sero-distribution of *Salmonella* isolated from various meat types at the Thai-Lao border area.

Table 2 Sero-distribution of *Salmonella* isolated from various meat types at the Thai-Lao border area.

***Salmonella* serotype**	**Nong Khai, Thailand**			**Vientiane, Laos PDR**			**Total**	
	Pork	**Chicken**	**Beef**	**Pork**	**Chicken**	**Beef**	**n**	**%**
S. Agona					2		2	0.86
S. Albany		1					1	0.43
S. Altona				2			2	0.86
S. Amsterdam				2		1	3	1.29
S. Anatum	3			2			5	2.16
S. Bareilly		3					3	1.29
S. Bovismorbificans			2				2	0.86
S. Brimingham				1			1	0.43
S. Brunei		1			1	3	5	2.16
S. Cerro			1				1	0.43
S. Corvallis		8	1	4	19		32	13.79
S. Duesseldorf		1					1	0.43
S. Eastbournc			2				2	0.86
S. Elisabethville						1	1	0.43
S. Enteritidis		1					1	0.43
S. Farehan			1				1	0.43
S. Farsta					1		1	0.43
S. Fulda			1			1	2	0.86
S. Gabon					1		1	0.43
S. Give	1			4			5	2.16
S. Goma				1			1	0.43
S. Havana				2			2	0.86
S. Hvittingfoss		2		2		1	5	2.16
S. Itami					2		2	0.86

S. Jerusalem	1						1	0.43
S. Kapemba					1		1	0.43
S. Kedougou				8	1		9	3.88
S. Kikoma						1	1	0.43
S. Kortrijk		1					1	0.43
S. Lexington			1				1	0.43
S. Livingstone					1		1	0.43
S. Lomita					1		1	0.43
S. London	2			32	1	26	61	26.99
S. Mbandaka		2			1		3	1.29
S. Meleagridis			1	3			4	1.72
S. Mikamasima	1						1	0.43
S. Monschui					3		3	1.29
S. Montevideo						1	1	0.43
S. Muenster	1					1	2	0.86
S. Newport		2		1			3	1.29
S. Ordonez		4					4	1.72
S. Planckendael				1			1	0.43
S. Regent					1		1	0.43
S. Rissen	5			3	3	4	15	6.47
S. Ruzizi			1				1	0.43
S. Saintpaul		2					2	0.86
S. Sangera						2	2	0.86
S. Stanley		1		1			2	0.86
S. Stanleyville		1					1	0.43
S. Typhimurium	3		1	2	3	2	11	4.74
S. Uganda		1					1	0.43
S. Wagenia						1	1	0.43
S. Weltevreden			6		1	7	14	6.03
Total	18	31	18	70	43	52	232	100.00

Table 3(on next page)

Variation of housekeeping genes loci of un-identified Sequence Type (ST) *Salmonella* strains circulating in the Thai-Lao border area.

Table 3 Variation of housekeeping genes loci of un-identified Sequence Type (ST) *Salmonella* strains circulating in the Thai-Lao border area.

Strain	**Most related ST**[a]	**7 Housekeeping gene for MLST**[b]						
		*aro*C	*dna*N	*hem*D	*his*D	*pur*E	*suc*A	*thr*A
LPO12	ST155	10	60	58	66	6→706	65	16
LPO58	ST29	16	16	20	18	8	12	18→930
LPO71	ST155	10	60	58	66	6→873	65	16
LPO76	ST7498	10	7	12	9	1176	9→925	2
LPO86	ST1541	197	187	10	234	8→6	65	22
LCH26	ST29	16	16	20	18	8	12	18→652
LCH50	ST469	92	107	79	156→985	64→84	151	87
LB03	ST155	10	60	58→98	66	6	65	16
LB11	ST29	16	16	20→10	18	8	12	18→140
LB13	ST1799	202→635	4→13	10	33→971	90	6	275→839
LB30	ST155	10	60	58	66	6	65	16→1241
LB31	ST5706	130	97	25	125→1370	84	9	970
LB46	ST446	15	126	101	88	8→880	19	18-2
LB49	ST155	10	60	58	66	6	65	16→604
LB60	ST64	10	14→491	15	31	25→235	20	33→631
LB68	ST365	130	97	25	125	84→309	9	16
TPO04	ST469	92	107	79	156	64→720	151	87
TPO07	ST34	10	19	12→758	9→578	5→620	9	2
TPO13	ST29	16	16	20	18	8→996	12	18
TPO17	ST3157	636	4→699	10	33	90→235	6→754	275
TPO23	ST469	92	107	79	156	64	151	87→631
TPO24	ST616	84	11→618	16	42	40	71	4→254
TCH02	ST197	197	187	10	234	8→898	844	22→723
TCH11	ST1541	197	187	10	234	8→996	65	22→844
TB04	ST29	16	16	20	18	8→996	12→487	18
TB12	ST283	101→30	97→760	25	86→1011	101→309	19→327	101
TB20	ST5706	130	97→955	25	125	84-309	9→327	970
TB21	ST1541	197	187	10	234→439	8→996	65	22

[a] The most genetic relateness Sequence Type (ST) with the untypable strain

[b] Variation of the housekeeping genes alleic number from known ST to untyable ST