1 **Epidemiological dynamic potential of transboundary *Salmonella* spp. in meat sold at**

2 **retail markets in the middle Mekong basin area**

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Abstract

Background: The surrounding areas of the middle Mekong basin, particularly along the border line between Thailand and Lao People's Democratic Republic (Lao PDR), ~~is~~ are considered to be a high-risk area for many livestock-associated foodborne illnesses, especially salmonellosis. ~~The aim of this study was~~is study aimed 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~~serotype characteristics, 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~~to enhance 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 the 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~~no evidence of shared genetic relatedness of *Salmonella* at retail sites among Thailand-Laos border zone. However, a diverse range of *Salmonella*~~their~~ genotypes had been spread over the area. Besides, the persistence of the residential pathogen and sharing of the supply route within-country can be inferred.

Conclusions: Regular disinfecting of all working areas a~~long with quality control checking at the pre-retail stage must be applied to reduce the transmission of *Salmonella* as well~~nd quality

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control checking at the pre-retail stage must be applied to reduce the transmission of *Salmonella* and other foodborne pathogens downstream consumers.

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

Introduction

Salmonella spp. is a~~n~~ ~~important~~significant causative agent of certain foodborne illnesses in humans and can be found ~~all over the world~~worldwide (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~~ significant 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~~is listed as the main factors influencing 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~~ 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~~production levels 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~~practice methods. Additionally, cultural preferences and an inadequate level of awareness among local people ~~with regard to~~about 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~~private and public vehicles to engage in trade and exchange various products. Therefore, the chances of pathogen transmissions between border-line communities are likely to be high.

In a study of *Salmonella* prevalence in the 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

99   that the prevalence of contaminated pork and beef in southern-Laos PDR was 93% and 82%,

100   respectively. Both studies reported a widespread variety of serotype distribution including *S.*

101   Typhimurium, *S*. Derby, *S*. Anatum or *S*. Rissen. Interestingly, almost all strains of *Salmonella*

102   are currently classified as multidrug-resistant strains. However, based on the data that has been

103   studied, there is a ~~clear~~n evident lack of reported information on poultry meat, while it is well

104   known that poultry meat is commonly consumed in this area. Additionally, ~~in order to gain~~

105   ~~access to in-depth epidemiological information, a study of the pathogen characteristics at the~~

106   ~~genetic level should be fulfilled in order~~to gain access to in-depth epidemiological information,

107   a study of the pathogen characteristics at the genetic level should be fulfilled to expand upon

108   the scope of epidemiological knowledge of these pathogens. Multilocus sequence typing

109   (MLST) technique is one of the methods employed in our study. This technique relies on ~~a~~

110   ~~comparison of the sequences of allele types in a specific group for each set of house-keeping~~

111   ~~gene~~comparing the sequences of allele types in a specific group for each set of house-keeping

112   genes ~~and focuses on the genetics in order~~s and focuses on the genetics to assemble data of the

113   sequence type (ST). The resulting findings can be compared with the information ~~presented in~~

114   ~~a number~~ of various databases related to the study of the global bacterial dynamic distribution

115   and their genetic evolution (Liu et al., 2011; Patchanee et al., 2017).

116   The purpose of the study was to investigate the prevalence of *Salmonella* in meat sold at retail

117   markets in the middle Mekong Basin area along the border of Thailand and Lao PDR,

118   surrounding the Thai-Lao Friendship Bridge I. Together with comparing the expressive and

119   characteristics (serotypes, ~~antimicrobial~~ antimicrobial-resistant patterns ~~and ST type) of~~

120   ~~*Salmonella* to expand upon the epidemiological knowledge of pathogen distribution~~, and ST

121   type) of Salmonella to expand upon pathogen distribution epidemiological knowledge. The

122   findings from this study would be ~~extremely usefu~~instrumental in ~~the creation of~~creating

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123 appropriate preventive measures that would help control the spread of human salmonellosis in

124 the region.

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126 **Materials and methods**

127 **Sample collection**

128 The sample size of this study was determined using the Win Epi online program

129 (http://www.winepi.net/uk/index.htm). For each parameter used, the degree of prevalence

130 identified in the previous study was used as the "expected prevalence"The degree of prevalence

131 identified in the previous study was used as the "expected prevalence" for each parameter used.

132 Rates of 65% (Sinwat et al., 2016), 73% (Trongjit et al., 2017) and 82% (Boonmar et al., 2013)

133 were chosen to calculate the sample sizes for pork, chicken and beef, respectively. Furthermore,

134 8.5% and 95% were selected as the "accepted error" and "confidence level" values,

135 respectively. for the required feature inputs. For the infinite population, a minimum of 121, 105

136 and 79 samples of pork, chicken and beef were designated, respectively. However, in order to

137 achieve even greater levels of accuracy and reliability, additional samples were carefully

138 chosen.

139 During the period of time from January to May, 2019, 370 samples (121, 133 and 116 samples

140 of pork, chicken and beef, respectively) were carefully collected from five retail markets in

141 Vientiane Capital, Lao PDR and six retail markets in Nong Khai Province, Thailand. All

142 samples were then individually labeled, put into plastic packs and stored in an icebox for

143 laboratory analysis within 24 hr at the Department of Veterinary Medicine, Faculty of

144 Agriculture, National University of Laos, Nabong Campus, Lao PDR.

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146 *Salmonella* **isolation and identification**

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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™ 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,

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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~~ multidrug-resistant.

Multilocus sequence typing (MLST)

Randomly selected 38 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 to 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

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A total of 370 meat samples were collected ~~during the period of~~from 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~~ compared 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.

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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.

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Commented [D22]: specify strains as coded alpha-numberics as in table 3

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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

270 the results might depend upon the time period of the sampling along with any existing

271 geographical factors. Nevertheless, high levels of *Salmonella* contamination still persist, even

272 though some intervention has been implemented in some of these areas. *Salmonella* remains a

273 problem in the meat being sold in this region, which has been an important public health

274 concern for the last half decade.

275 The prevalence of *Salmonella* in the Laos PDR-originated samples was statistically and

276 significantly high. Normally, sanitation practices are different at each location. In fact, there is

277 no supermarket in Laos DPR. All fresh food, such as meat, can only be bought from fresh

278 markets and mini-grocery stores. The lack of covering materials, unsuitable storage conditions

279 and inadequate disinfection practices at the purchasing areas during the meat cutting and

280 handling processes can substantially increase the risk of bacterial colonization (Gomes-Neves

281 et al., 2012; Patchanee et al., 2016). In Thailand, supermarkets are the preferred place to

282 purchase meat. One-sixth of the Thai samples collected in our study were obtained from

283 supermarkets. It is known for stringently regulating their facilities, as well as for implementing

284 biosecurity and hygienic policies along with quality control practices at the pre-harvesting and

285 harvesting stages along the supply chain. However, in general, standard protocols for every

286 type of retail outlet (supermarkets, mini-grocery stores and fresh markets) tend to be higher in

287 bigger cities (Trongjit et al., 2017). With a focus on meat type, pork was found to be the most

288 prevalent when compared with the other types of meat. The reason for this may not be clear. It

289 could be due to the high bacterial loads that emerge during previous production stages, which

290 can then lead to instances of contamination at retail outlets. According to the information

291 obtained from previous studies, *Salmonella* prevalence at pig farms and during pig slaughtering

292 processes was higher than in chicken and beef production (Padungtod & Kaneene, 2006;

293 Trongjit et al., 2017; Phongaran et al., 2019). Another possible explanation for this is that pork

294 is the most common type of meat consumed in this region of study (Napasirth & Napasirth,

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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~~several serotypes have been detected, 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~~about the isolation of *Salmonella* in the region. Time factor and sample picking~~, as well as~~ and the ~~cross~~ 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~~represent 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~~

320 critical factor that has led to the current situation of resistance (Jajere, 2019). On the contrary,

321 low resistance rates have been recorded for cefotaxime, norfloxacin and ciprofloxacin.

322 Instances of resistance should be of particular concern in resource-limited countries because

323 these are the drugs of choice that are recommended to treat human salmonellosis. Particularly

324 for quinolones (norfloxacin and ciprofloxacin), the antimicrobial class is now often used as the

325 first line of treatment (Kurtz et al., 2017). Of course, all of the quinolone-resistant strains

326 discovered in these study areas originated from chicken meat collected in Thailand, and all

327 were found to be resistant to 7-9 of the tested antimicrobials. Selective pressures such as those

328 associated with antimicrobial use, unsuitable temperatures or pH levels, could be considered

329 sub-lethal stress factors during the short production cycle in broiler farms. Additionally, the

330 chicken's gut ecology should be taken into account when developing resistance to bacterial

331 pathogens (Shang et al., 2018; Yang et al., 2019).

332 An overview of the phylogenetic tree reveals that most of the strains obtained in this study

333 were diverse. Five-sixths of all strains could not be named to any ST. Microevolution or

334 mutation is a possible explanation for the finding (Harbottle et al., 2006; Liu et al., 2011). This

335 may be proven if some unidentified strains differed in only one or two loci from the other

336 known nomenclatures. Moreover, the variation of 3-5 housekeeping genes of known related

337 ST were also denoted. Therefore, of those, many strains cannot be grouped in any clonal

338 complexes. Those that were unidentified may be newly detected STs that have not yet been

339 recorded in the MLST database.

340 At this moment, shared genetical relatedness of *Salmonella* among Thai-Laos border area was

341 not evidenced. The finding infers that the transboundary food supply chain from locations

342 ahead of the marketplace (farm and/or slaughterhouse) are not involve to contamination

343 occursis not involved in contamination. However, since only 38 strains were analysed, it cannot

344 100% prove that *Salmonella* contamination from both countries are not related. An analysis

345 ~~together with information of all geographical matching strains previously submitted in MLST~~

346 ~~database should be recommended fulfilled the gap, further~~and information of all geographical

347 matching strains previously submitted in MLST database should be recommended to fulfil the

348 gap. Interestingly, various specimens of the *Salmonella* genotypes collected from a single

349 market were remarked. In details, 9 and 6 *Salmonella* genotypes were detected in various meat

350 types collected from Phonsavang and Chengsavang markets, respectively. These findings

351 evidence that more than one infection source can exist at a given location. Contamination can

352 occur by itself in the purchasing area or as a result of inadequate processing when the meat

353 product may have already been contaminated. Unhygienic practices at previous production

354 sites, such as on farms and at slaughterhouses, or at transportation hubs may be causes of

355 transmission in the present situation (Heyndrickx et al., 2002; Campioni et al., 2012).

356 According to the ~~findings of the ST155 and ST365 clonal strain~~ST155 and ST365 clonal

357 strains' finding~~s~~, both were detected on different sampling dates from the same marketplaces.

358 Persistence of residential *Salmonella* flora could therefore be inferred. Moreover, two strains

359 of ST469 were recovered from different markets ~~at~~ in nearby areas. Consequently, the sharing

360 of supply routes at the nearby areas could play a significant role in ~~the dissemination~~

361 ~~of~~disseminating *Salmonella* spp.

362

363 **Conclusions**

364 As has been demonstrated, ~~the presence of *Salmonella*~~Salmonella's presence in meat sold in

365 the middle Mekong basin area was relatively high in terms of prevalence. Standard hygienic

366 protocols are known to be maintained at a higher level in administrative areas. The findings

367 from the molecular MLST indicate that extensive quality control checking at pre-retail stages

368 should be implemented. Furthermore, regular disinfecting of all equipment, as well as at

369 working areas, must be applied. Future efforts in ~~the strengthening of food safety education~~

370 ~~and awareness programs would help authorities to~~strengthening food safety education and

371 awareness programs would help authorities establish strategies that could potentially reduce

372 the transmission of *Salmonella* and other foodborne pathogens to downstream consumers.

373

Acknowledgements

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).

> **Commented [D28]:** Rewrite figure titles and legends carefully making them descriptive
>
> **Commented [D29]:** Percent of what?

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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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