Molecular evidence for cross boundary spread of *Salmonella* spp. in meat sold at retail markets in the middle Mekong basin area (#53241)

First revision

Guidance from your Editor

Please submit by **18 Feb 2021** for the benefit of the authors .

Structure and Criteria
Please read the 'Structure and Criteria' page for general guidance.

Custom checks
Make sure you include the custom checks shown below, in your review.

Raw data check
Review the raw data.

Image check
Check that figures and images have not been inappropriately manipulated.

Privacy reminder: If uploading an annotated PDF, remove identifiable information to remain anonymous.

Files
Download and review all files from the materials page.

1 Tracked changes manuscript(s)
1 Rebuttal letter(s)
2 Figure file(s)
5 Table file(s)
1 Raw data file(s)

⚠ Custom checks

DNA data checks

Have you checked the authors data deposition statement?

Can you access the deposited data?

Has the data been deposited correctly?

Is the deposition information noted in the manuscript?

Structure and Criteria

Structure your review

The review form is divided into 5 sections. Please consider these when composing your review:

1. **BASIC REPORTING**
2. **EXPERIMENTAL DESIGN**
3. **VALIDITY OF THE FINDINGS**
4. General comments
5. Confidential notes to the editor

🗋 You can also annotate this PDF and upload it as part of your review

When ready [submit online](#).

Editorial Criteria

Use these criteria points to structure your review. The full detailed editorial criteria is on your [guidance page](#).

BASIC REPORTING

- Clear, unambiguous, professional English language used throughout.
- Intro & background to show context. Literature well referenced & relevant.
- Structure conforms to [PeerJ standards](#), discipline norm, or improved for clarity.
- Figures are relevant, high quality, well labelled & described.
- Raw data supplied (see [PeerJ policy](#)).

EXPERIMENTAL DESIGN

- Original primary research within [Scope of the journal](#).
- Research question well defined, relevant & meaningful. It is stated how the research fills an identified knowledge gap.
- Rigorous investigation performed to a high technical & ethical standard.
- Methods described with sufficient detail & information to replicate.

VALIDITY OF THE FINDINGS

- ℹ Impact and novelty not assessed. Negative/inconclusive results accepted. *Meaningful* replication encouraged where rationale & benefit to literature is clearly stated.
- All underlying data have been provided; they are robust, statistically sound, & controlled.
- Speculation is welcome, but should be identified as such.
- Conclusions are well stated, linked to original research question & limited to supporting results.

Standout reviewing tips

The best reviewers use these techniques

Tip	*Example*
Support criticisms with evidence from the text or from other sources	*Smith et al (J of Methodology, 2005, V3, pp 123) have shown that the analysis you use in Lines 241-250 is not the most appropriate for this situation. Please explain why you used this method.*
Give specific suggestions on how to improve the manuscript	*Your introduction needs more detail. I suggest that you improve the description at lines 57- 86 to provide more justification for your study (specifically, you should expand upon the knowledge gap being filled).*
Comment on language and grammar issues	*The English language should be improved to ensure that an international audience can clearly understand your text. Some examples where the language could be improved include lines 23, 77, 121, 128 – the current phrasing makes comprehension difficult.*
Organize by importance of the issues, and number your points	*1. Your most important issue* *2. The next most important item* *3. ...* *4. The least important points*
Please provide constructive criticism, and avoid personal opinions	*I thank you for providing the raw data, however your supplemental files need more descriptive metadata identifiers to be useful to future readers. Although your results are compelling, the data analysis should be improved in the following ways: AA, BB, CC*
Comment on strengths (as well as weaknesses) of the manuscript	*I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.*

Molecular evidence for cross boundary spread of *Salmonella* spp. in meat sold at retail markets in the middle Mekong basin area

Dethaloun Meunsene [1] , **Prapas Patchanee** [2] , **Ben Pascoe** [3] , **Phacharaporn Tadee** [4] , **Pakpoom Tadee** [Corresp. 2]

[1] Graduate Program in Veterinary Science, Faculty of Veterinary Medicine, Chiang Mai University, Muang, Chiang Mai, Thailand

[2] Integrative Research Center for Veterinary Preventive Medicine, Faculty of Veterinary Medicine, Chiang Mai University, Muang, Chiang Mai, Thailand

[3] The Milner Centre for Evolution, University of Bath, Claverton Down, Bath, United Kingdom

[4] Faculty of Animal Science and Technology, Maejo University, San Sai, Chiang Mai, Thailand

Corresponding Author: Pakpoom Tadee
Email address: pakpoom.t@cmu.ac.th

Background: The surrounding areas of the middle Mekong basin , particularly along the border line between Thailand and Lao People's Democratic Republic (Lao PDR), are high-risk areas for many livestock-associated foodborne illnesses, especially salmonellosis. This study aimed to determine the prevalence and characteristics of *Salmonella* spp. contamination 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. We focused on the prevalent serotypes, antimicrobial susceptibility profiles and the multilocus sequence type (MLST) genotypes of the collected *Salmonella* strains. **Results:** From a total of 370 meat samples collected, 63% were 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 no evidence of shared genetic relatedness of *Salmonella* at retail sites among Thailand-Laos border zone. However, a diverse range of *Salmonella* genotypes were spread over the area. Besides, the persistence of the residential pathogen and sharing of the supply route within-country can be inferred. **Conclusions:** Given the high levels of contamination of retail meats, regular disinfecting of all working areas and quality control checking at pre-retail stage must be applied to reduce the transmission of *Salmonella* and other foodborne pathogens to consumers. The findings of this study will make a significant contribution to the current understanding of *Salmonella* epidemiology to enhance food security in the region.

1 **Molecular evidence for cross boundary spread of *Salmonella* spp. in meat sold at retail**

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

3

4 Dethaloun Meunsene[1], Prapas Patchanee[2], Ben Pascoe[2,3], Phacharaporn Tadee[4], Pakpoom

5 Tadee[2*]

6

7 [1]*Graduate Program in Veterinary Science, Faculty of Veterinary Medicine, Chiang Mai*

8 *University, Chiang Mai, 50100, Thailand*

9 [2]*Integrative Research Center for Veterinary Preventive Medicine, Faculty of Veterinary*

10 *Medicine, Chiang Mai University, Chiang Mai, 50100, Thailand*

11 [3]*The Milner Centre for Evolution, University of Bath, Claverton Down, Bath, BA2 7AY, United*

12 *Kingdom*

13 [4]*Faculty of Animal Science and Technology, Maejo University, Chiang Mai,50290, Thailand*

14

15 *** Corresponding author:** Pakpoom Tadee

16 Integrative research center for Veterinary Preventive Medicine, Faculty of Veterinary Medicine,

17 Chiang Mai University, Mae Hia, Muang, Chiang Mai, 50100, THAILAND. Tel: +66 53

18 948023, Fax: +66 53 948062, E-mail: pakpoom.t@cmu.ac.th

19

20

21

22

23

24

25

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), are high-risk areas for

many livestock-associated foodborne illnesses, especially salmonellosis. This study aimed to

determine the prevalence and characteristics of *Salmonella* spp. contamination 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. We focused on the prevalent serotypes,

antimicrobial susceptibility profiles and the multilocus sequence type (MLST) genotypes of the

collected *Salmonella* strains.

Results: From a total of 370 meat samples collected, 63% were 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 no evidence of shared genetic

relatedness of *Salmonella* at retail sites among Thailand-Laos border zone. However, a diverse

range of *Salmonella* genotypes were spread over the area. Besides, the persistence of the

residential pathogen and sharing of the supply route within-country can be inferred.

Conclusions: Given the high levels of contamination of retail meats, regular disinfecting of all

working areas and quality control checking at pre-retail stage must be applied to reduce the

transmission of *Salmonella* and other foodborne pathogens to consumers. The findings of this

47 study will make a significant contribution to the current understanding of *Salmonella*

48 epidemiology to enhance food security in the region.

49 **Keywords:** *Salmonella*; retail market; serotype; antimicrobial resistant; MLST; Thailand-Laos

50 border; middle Mekong basin

51 **Introduction**

52 *Salmonella* spp. is a significant causative agent of certain foodborne illnesses in humans and can

53 be found worldwide (Sirichote et al., 2010; Van Boxstael et al., 2012; Basler et al., 2016).

54 Annually, ninety million cases resulting in 150,000 deaths among salmonellosis patients have

55 been recorded (Campioni et al., 2012). Along with the direct effects of *Salmonella* spp. have on

56 the gastrointestinal tract, evidence of drug resistance is also a major public health concern (Foley

57 & Lynne, 2008; Kurtz et al., 2017; Jajere, 2019). This can result in a reduction in the

58 effectiveness of first line empirical treatments and limit treatment choices (Van Boxstael et al.,

59 2012).

60 Livestock products (farm animal-origin food), especially meats, are an important source of

61 human salmonellosis (Heyndrickx et al., 2002; Mainali et al., 2009; Rostagno & Callaway,

62 2012). Retail markets have been identified as the most significant point of contact for

63 salmonellosis exposure and transmission among humans (Hauser et al., 2011; Gomes-Neves et

64 al., 2012). Improper management and biosecurity during the production process, such as on

65 farms or in slaughterhouses also contribute to the risk of increased pathogen loads in retail meats.

66 Contamination can occur directly or through contaminated equipment due to improper handling

67 practices or unsuitable storage conditions (Lo Fo Wong et al., 2002).

68 Increased demand for meat consumption has led to an intensive transformation of the animal

69 production industry (Guardabassi et al., 2008). Good Management Practices (GMP) must be

70   implemented at all production levels to ensure food safety for consumers. However, these

71   practices cannot be implemented 100% in certain developing regions, such as the middle

72   Mekong basin and the surrounding areas, particularly along the border between Thailand and

73   Lao People's Democratic Republic (Lao PDR). Animal farming practices are being used

74   extensively in traditional free-range and smallholder backyard systems, which often employ the

75   minimum hygienic sanitation systems. Moreover, inadequate practices in slaughterhouses and

76   retail outlets, such as on-floor slaughtering and uncontrolled storage conditions in purchasing

77   areas are not ideal practices. Cultural preferences and a lack of awareness among local people

78   about raw meat consumption, in addition to an absence of reliable and high-quality resources,

79   such as clean water and cooking supplies are also important risk factors to be considered

80   (Wilson, 2007; Conlan et al., 2014; Okello et al., 2017). Taken together, these risk factors

81   increase the opportunity for several foodborne diseases, including salmonellosis (Mughini-Gras

82   et al., 2014; Ferrari et al., 2019).

83   In 1994, the Thai-Lao Friendship Bridge I linking Nong Khai Province, Thailand and the

84   Vientiane Capital of Lao PDR was officially opened. More than three million people cross the

85   bridge each year (Australian Embassy, Thailand, 2011). These people cross by way of private

86   and public vehicles to engage in trade and exchange various products. Therefore, the chances of

87   pathogen transmissions between borderline communities are likely to be high.

88   In a study of *Salmonella* prevalence in the meat being sold surrounding the Thailand-Laos border

89   region, Sinwat et al., (2016) reported that the prevalence of pork contamination in border areas of

90   Thailand and Laos PDR was 65%. Moreover, Boonmar et al., (2013) reported that the prevalence

91   of contaminated pork and beef in southern-Laos PDR was 93% and 82%, respectively. Both

92   studies reported a widespread variety of serotype distribution including *S.* Typhimurium, *S.*

93   Derby, *S.* Anatum and *S.* Gisen. Interestingly, almost all strains of *Salmonella* are currently

94   classified as multidrug-resistant strains. However, based on the data that has been studied, there

95   is an evident lack of reported information on poultry meat, while it is well known that poultry

96   meat is commonly consumed in this area. Additionally, to gain access to in-depth

97   epidemiological information, a study of the pathogen characteristics at the genetic level should

98   be fulfilled to expand upon the scope of epidemiological knowledge of these pathogens.

99   Multilocus sequence typing (MLST) technique is one of the methods employed in our study.

100   This technique relies on a comparison of the sequences of allele types in a specific group for

101   each set of house-keeping gene and focuses on the genetics in order to assemble data of the

102   sequence type (ST). The resulting findings can be compared with the information of various

103   databases related to the study of the global bacterial dynamic distribution and their genetic

104   evolution (Liu et al., 2011; Patchanee et al., 2017; Zhou et al., 2020).

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

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

107   surrounding the Thai-Lao Friendship Bridge I. We compare strain serotypes, genotypes and their

108   antimicrobial-resistance patterns and identify genetic and phenotypic variation in *Salmonella*

109   strains. Our findings inform regional knowledge gaps in the epidemiology of *Salmonella* and

110   form the basis for appropriate preventive measures to help control the spread of human

111   salmonellosis in the region.

112

113   **Materials and methods**

114   **Sample collection**

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

116   (http://www.winepi.net/uk/index.htm). The prevalence rates identified in previous studies of

117   65% (Sinwat et al., 2016), 73% (Trongjit et al., 2017) and 82% (Boonmar et al., 2013) were

118   chosen as the "expected prevalence" to calculate the sample sizes for pork, chicken and beef,

119   respectively. An accepted error rate of 8.5% and 95% confidence levels were selected for the

120   required feature inputs. For an infinite population, a minimum of 121, 105 and 79 samples of

121   pork, chicken and beef were designated, respectively. However, in order to achieve greater levels

122   of accuracy and reliability, additional samples were carefully chosen.

123   During the period of January to May 2019, 370 samples (121, 133 and 116 samples of pork,

124   chicken and beef, respectively) were carefully collected from five retail markets in Vientiane

125   Capital, Lao PDR and six retail markets in Nong Khai Province, Thailand. All samples were

126   individually labeled, put into plastic packs and stored in an icebox for laboratory analysis within

127   24 hr at the Department of Veterinary Medicine, Faculty of Agriculture, National University of

128   Laos, Nabong Campus, Lao PDR.

129

130   ***Salmonella* isolation and identification**

131   Isolation and identification of *Salmonella* spp. from meat samples (pork, chicken, beef) were

132   performed following ISO6579: 2002 Amendment 1:2007, Annex D technique. Accordingly, 25 g

133   of samples were enriched with 225 ml of Buffered Peptone Water (BPW; Merck, Germany). The

134   mixing materials were homogenized for 120 sec and incubated at 37ºC for 24 hr. An aliquot of

135   100 µl of the pre-enriched inoculum was transferred to 10 ml of Rappaport-Vassiliadis broth

136   (RV; Oxoid, UK) at 42ºC for an incubation period of 24 hr. The likely colonies were then

137   streaked on Xylose-Lysine-Desocholate agar (XLD; Oxoid, UK) and Brilliant-green Phenol Red

138   Lactose sucrose agar (BPLS; Merck, Germany). After an incubation period of 24 hr at 37ºC, the

139   presumptive colonies were then placed on Triple Sugar Iron agar (TSI; Oxoid, England), urease

140   and Motility Indole-Lysine agar (MIL; Merck, Germany) for bio-chemical confirmation. An

141   analysis of the strains indicated a correct bio-chemical reaction as positive for *Salmonella*.

142   Finally, all results were then recorded.

143

144   **Serotyping and Antimicrobial susceptibility testing**

145   All detected *Salmonella* spp. specimens were serotyped using the serum-agglutination test

146   according to the White-Kauffmann-Le Minor scheme (Popoff et al., 1993), and were also run

147   through antimicrobial susceptibility testing with agar disk diffusion using ten panels of

148   antimicrobial agents. Amoxicillin-clavulanic acid (AMC) 20/10 µg, ampicillin (AMP) 10 µg,

149   chloramphenicol (C) 30 µg, ciprofloxacin (CIP) 5 µg, cefotaxime (CTX) 30 µg, nalidixic acid

150   (NA) 30 µg, norfloxacin (NOR) 10 µg, streptomycin (S) 10 µg, sulfamethoxazole-trimethoprim

151   (SXT) 23.75/1.25 µg and tetracycline (TE) 30 µg (CLSI, 2011) were included. Strains that

152   existed with intermediate resistance were grouped as being susceptible in order to avoid

153   overestimation. Strains that resisted ≥ 3 of the antimicrobial agents were considered multidrug-

154   resistant.

155

156   **Multilocus sequence typing (MLST)**

157   Randomly selected 38 specimens of *Salmonella* obtained from the most frequently found

158   serotypes were genotyped using the MLST technique. DNA was extracted according to the

159   protocol described by Liu et al., (2011). Seven housekeeping genes, including *aro*C (chorismate

160   synthase); *dna*N (DNA polymerase III beta subunit); *hem*D (uroporphyrinogenIII cosynthase);

161   *pur*E (phosphoribosylaminoimidazole carboxylase); *suc*A (alpha ketoglutarate dehydrogenase);

162   *his*D (histidinol dehydrogenase) and *thr*A (aspartokinase I/homoserine dehydrogenase), were

163   selected for MLST profiling (REF). PCR amplification of all 7 genes was accomplished using

164   the method previously described by Kotetishvili et al., (2002). In brief, the PCR amplification

165   conditions were 94°C for 5 min, followed by 35 amplification cycles, each consisting of

166   sequential incubation at 94°C (45 s), 55°C (45 s), and 72°C (5 min). Subsequently, the products

167   were sent to be sequenced at the Macrogen Service Center, Republic of Korea.

168   The sequences obtained in each gene **(Supplementary file 1** and **Table S1)** were transformed

169   into allele numbers, and were compiled according to the sequence type (ST) data obtained from

170   the http://enterobase.warwick.ac.uk/species/senterica/allele_st_search database. Finally, all data

171   of the *Salmonella* strains acquired from this study were analysed. A phylogenetic tree was

172   constructed using Bionumerics® software version 7.6 (Applied Maths, Belgium).

173

174   **Statistical analysis**

175   *Salmonella* prevalence with their 95% confidence level were determined by descriptive

176   statistical analysis. The comparison of *Salmonella* positive proportion among their relevant

177   specifications (location or meat type) were considered using fisher's exact test. Epi Info™

178   version 7 completed all analyses. Statistically significant levels were determined at $p < 0.05$.

179

180   **Results**

181   A total of 370 meat samples were collected between January and May 2019. This included 135

182   samples from six retail markets in Nong Khai Province, Thailand and a further 235 samples from

183   five retail markets in Vientiane Capital, Lao PDR. Samples were collected from three different

184   types of meat, composed of samples from pork (*n*=121), chicken (*n*=133) and beef (*n*=116).

185   The overall prevalence of *Salmonella* spp. was found to be 62.70% (232/370; 95% CI: 57.67-

186   67.48%). Prevalence rates were significantly higher in the samples collected from Laos PDR

187   (70.21%; 165/235) compared to those collected from the Thai sampling sites (49.63%; 67/135)

188   (p<0.05). *Salmonella* spp. prevalence was highest in pork (72.73%; 88/121) compared to chicken

189   (55.64%; 74/133) and beef (60.34%; 70/116). The higher rate samples recovered from pork was

190   statistically significant when compared to those recovered from chicken meats (p<0.05), but

191   there was less confidence in differences in sample recovery from beef (p=0.05). **Table 1**

192   demonstrates the distribution details of *Salmonella* spp. positives with 95% confidence intervals

193   among the different locations and sample types.

194   In total, 53 *Salmonella* spp. serotypes were identified by serum-agglutination according to the

195   White-Kauffmann-Le Minor scheme (**Table 2**). The most common serotypes were *S.* London

196   (26.99%; 61/232), followed by *S.* Corvallis (13.79%; 32/232), *S.* Rissen (6.47%; 15/232) and *S.*

197   Weltevreden (6.03%; 14/232), respectively. These four serotypes were common in both

198   countries, with the exception of *S.* London which was only identified twice in the Thai samples.

199   Stratifying for each meat type, *S.* London was the most common serotype detected from the pork

200   (*n*=34) and beef samples (*n*=26). For the chicken samples, the highest degree of frequency was

201   found for *S.* Corvallis (*n*=27). Interestingly, there were just three serotypes, *S.* London, *S.* Rissen,

202   *S.* Corvallis and *S.* Typhimurium, that were distributed among all meat types.

203   All 232 *Salmonella* strains were submitted for antimicrobial susceptibility testing. Consequently,

204   76 of them (32.75%) were classified as multidrug resistant strains. Resistance to tetracycline

205   (49.57%; 115/232) was found in the highest frequency, followed by ampicillin (35.34%; 82/232),

206 and sulfamethoxazole-trimethoprim (28.45%; 66/232), respectively. On the other hand, 94

207 strains (40.52%) were found to be susceptible to all of the tested antimicrobials. Additionally,

208 only 3 strains (1.29%) were found to be resistant to cefotaxime, while 5 (2.16%) and 6 (2.59%)

209 strains were found to be resistant to norfloxacin and ciprofloxacin, respectively. In Laos PDR,

210 rates of resistance were also highest against tetracycline (57.78%; 95/165), ampicillin (34.55%;

211 57/165) and sulfamethoxazole-trimethoprim (32.12%; 53/165). In Thailand, the resistant rates

212 were ranked as follows; ampicillin (37.31%; 25/67), tetracycline (29.85%; 20/67) and

213 streptomycin (20.90%; 14/67) (**Fig. 1**). Resistance rates differed between meat sources,

214 especially in the most effective antimicrobials, for instance almost all strains that were resistant

215 against norfloxacin or ciprofloxacin were from chicken meat (**Fig. 1**). Thus related with the

216 individual data, there were three strains listed at least seven antimicrobials test (TCH69: AMP /

217 AMC / CIP / NA / NOR / SXT / TE, TCH44: AMP / AMC / C / CIP / NA / NOR / SXT / TE and

218 TCH32: AMP / AMC / C / CIP / NA / NOR / S / SXT / TE) (**Table S2**), all three were isolated

219 from Nong Khai, Thailand.

220 **Fig. 2** demonstrated the genetic relatedness of *Salmonella* currently being detected at the

221 Thailand-Laos border area. From the 38 *Salmonella* strains analysed, 35 genetic characters were

222 found. Many of the strains (*n*=28) could not be assigned to a known ST and 11 of them could not

223 be grouped in any previously identified ST- clonal complex. One to five variant of

224 housekeeping genes of those 28 un-identified from known ST were displayed in **Table 3**. For

225 example, in the details, strain LPO12 with the closest of ST155 sequence type demonstrated the

226 variation of *pur*E gene to the allelic number 706. Besides, strain LCH50 with the closest of

227 ST469 demonstrated the variation of *his*D and *pur*E to the allelic number of 985 and 84,

228 respectively. However, for the 7 known STs (ST64, ST155, ST365, ST469, ST516, ST1541,

229   ST5706), four of those (ST64, ST516, ST1541, ST5706) were distinct to a single strain.

230   Nonetheless, two strains were grouped in the ST155, ST365 and ST 469. From the displayed in

231   **Fig. 2**, strains grouped in ST155 were derived from a Lao-Aussi market, one (LCH18; 16 Mar)

232   was chicken meat and the another (LPO42; 8 Apr) was pork. Two strains of ST365 originated

233   from Phonsavang market, one (LCH23; 16 Mar) was chicken and the another (LB58; 20 May)

234   was beef were also identified. Additionally, the strains grouped in ST469 were originated from

235   pork (LPO01; 2 May) and beef (LB29; 16 Mar) samples collected from different markets located

236   in nearby areas.

237

238   **Discussion**

239   The findings obtained from this study represent scientific information on the burden and

240   intensity of *Salmonella* in livestock meats in a 30 km radius surrounding the Thai-Lao Friendship

241   Bridge I. It is well known that this bridge is the primary place for the expansion of trade that has

242   occurred along the Thailand and Laos PDR border since 1994. Overall, prevalence of *Salmonella*

243   spp. in all sampled meat were found to be 63%. Pork had the highest prevalence (73%), followed

244   by beef (60%) and just over half of the chicken meats (56%) tested were contaminated with

245   *Salmonella*. Contamination of pork was higher than previously observed in a similar study by

246   Sinwat et al., (2016), where 65% (95% CI: 59.26-70.47%) of pork were contaminated. However,

247   for beef samples the degree of prevalence in this study was lower than the results of a study

248   conducted by Boonmar et al., (2013), in which the prevalence was recorded at 82% (95% CI:

249   58.97-93.81%). No studies have reported on the prevalence of *Salmonella* contamination in

250   chicken meats sold in this region. A study conducted by Trongjit et al., (2017) reported on the

251   degree of prevalence of *Salmonella* infection at the Thai-Cambodia border, which was found to

252   be 73.43% (95% CI: 66.65-79.42%), which is higher than our observed results. In general, most

253   studies have not demonstrated much difference when reporting numbers, which has been proven

254   by an overlap in the 95% confidence intervals. Variations in the results might depend upon the

255   time period of the sampling along with any existing geographical factors. Nevertheless, high

256   levels of *Salmonella* contamination still persist, even though some intervention has been

257   implemented in some of these areas. *Salmonella* remains a problem in the meat being sold in this

258   region, which has been an important public health concern for the last half decade.

259   The samples collected from meat sold in Laos PDR were statistically and significantly higher

260   than those collected over the border in Thailand **(Table 1)**. Normally, sanitation practices are

261   different at each location. In fact, there is no supermarket in Laos DPR. All fresh food, such as

262   meat, can only be bought from fresh markets and mini-grocery stores. The lack of covering

263   materials, unsuitable storage conditions and inadequate disinfection practices at the purchasing

264   areas during the meat cutting and handling processes can substantially increase the risk of

265   bacterial colonization (Gomes-Neves et al., 2012; Patchanee et al., 2016). In Thailand,

266   supermarkets are the preferred place to purchase meat. One-sixth of the Thai samples collected

267   in our study were obtained from supermarkets. Supermarkets provide stringent regulation of their

268   facilities and implement biosecurity and hygiene policies along with quality control practices at

269   the pre-harvesting and harvesting stages along the supply chain. However, in general, standard

270   protocols for every type of retail outlet (supermarkets, mini-grocery stores and fresh markets)

271   tend to be higher in larger cities (Trongjit et al., 2017).

272   With a focus on meat type, pork was found to be the most prevalent when compared with the

273   other types of meat. The reason for this may not be clear. It could be due to the high bacterial

274   loads that emerge during previous production stages, which can then lead to instances of

275   contamination at retail outlets. According to the information obtained from previous studies,

276   *Salmonella* prevalence at pig farms and during pig slaughtering processes was higher than in

277   chicken and beef production (Padungtod & Kaneene, 2006; Trongjit et al., 2017; Phongaran et

278   al., 2019). Another possible explanation for this is that pork is the most common type of meat

279   consumed in this region (Napasirth & Napasirth, 2018). The amount of dressing required, along

280   with any other forms of manipulation before meat is sold, would likely increase the opportunity

281   for contamination or re-contamination by product exposure at the final.

282   Based on the findings of previous studies, *S.* Typhimurium and *S.* Rissen are the majority

283   serotypes identified in pork (Patchanee et al., 2016; Sinwat et al., 2016). For chicken meat, *S.*

284   Corvallis and *S.* Enteritidis are known to be the dominant serotypes (Trongjit et al., 2017).

285   Additionally, *S.* Stanley and *S.* Typhimurium have been reported as the most commonly recorded

286   serotypes in beef (Boonmar et al., 2013). At the moment, these serotypes have not been

287   universally matched to each meat type, but low frequencies were recorded in some instances.

288   However, *S.* Corvallis is still noted as being the dominant serotype in chicken meat **(Table 2)**.

289   Consequently, new typical sero-characteristics for this region should be set for pork and beef. As

290   the data indicates, *S.* London is presently the most common serotype. Furthermore, several

291   serotypes have been detected, such as *S.* Altona, *S.* Cerro, *S.* Elisabethville, *S.* Itami, *S.*

292   Mikamasima, *S.* Ruzizi, etc. They have been reported for the very first time about the isolation of

293   *Salmonella* in the region. Time factor and sample picking and the cross-contamination that

294   occurs from other sources, are notable factors. Furthermore, even though the same serotype

295   might be presented at several meat origins, it cannot be concluded that two or three meat types

296   would represent a sharing pool for *Salmonella* identified from similar sources (Sinwat et al.,

297   2016). All of which would need to be proven.

298   *Salmonella* specimens isolated in this study display a relatively high frequency of resistance

299   against tetracycline and ampicillin **(Fig. 1)**, which is consistent with the findings of previous

300   investigations in the region (Padungtod & Kaneene, 2006; Pulsrikarn et al., 2012; Boonmar et

301   al., 2013). From the past until now, the antimicrobials have been widely used for treatment and

302   prophylaxis in livestock. However, excessive or inappropriate use is considered to be a critical

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

304   resistance rates have been recorded for cefotaxime, norfloxacin and ciprofloxacin. Instances of

305   resistance to these antimicrobials are particularly important as these are the drugs of choice for

306   treatment of human salmonellosis. Specifically, quinolones (norfloxacin and ciprofloxacin) are

307   now often used as the first line of treatment (Kurtz et al., 2017). All quinolone-resistant strains

308   isolated in these study areas originated from chicken meat collected in Thailand, and all were

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

310   associated with antimicrobial use, unsuitable temperatures or pH levels, could be considered sub-

311   lethal stress factors during the short production cycle in broiler farms. Additionally, the

312   characterizing on bacterial community composition in chicken's gut should be taken to

313   determine the mechanisms of action on the resistome (Shang et al., 2018; Yang et al., 2019).

314   The strains collected in this study were genetically diverse and five-sixths could not be assigned

315   to a previously described ST. Regional diversity following microevolution or mutation is a

316   possible explanation for the finding (Harbottle et al., 2006; Liu et al., 2011). Variation in 3-5 of

317   the housekeeping genes used to determine ST was also observed, meaning that those strains

318   could not be assigned to any clonal complex either. Large scale, shared genetic relatedness of

319   *Salmonella* strains collected along the Thai-Lao border was not observed **(Fig. 2)**. The finding

320   infers that the transboundary food supply chain from locations ahead of the marketplace (farm

321 and/or slaughterhouse) is not involved in contamination. A larger scale analysis, including all

322 strains previously collected in the region and deposited in MLST databases would help identify

323 additional shared reservoirs of contamination that may have been missed in our study.

324 *Salmonella* genotypes collected from a single market also demonstrated high levels of diversity,

325 nine and six *Salmonella* genotypes were isolated from various meat types in markets from

326 Phonsavang and Chengsavang, respectively. This suggests that more than one infection source

327 can exist at a given location. Contamination can occur by itself in the purchasing area or as a

328 result of inadequate processing when the meat product may have already been contaminated.

329 Unhygienic practices at previous production sites, such as on farms and at slaughterhouses, or at

330 transportation hubs may increase transmission (Heyndrickx et al., 2002; Campioni et al., 2012).

331 Clones of sequence types ST155 and ST365 were detected on different sampling dates from the

332 same marketplaces, indicative of persistent or residential *Salmonella* contamination.

333 Additionally, two strains of ST469 were recovered from different markets in nearby areas,

334 hinting at potential shared supply routes and their role in dissemination of *Salmonella*.

335

336 **Conclusions**

337 As has been demonstrated, *Salmonella*'s presence in meat sold in the middle Mekong basin area

338 was relatively high in terms of prevalence. Standard hygienic protocols are known to be

339 maintained at a higher level in administrative areas. The findings from the molecular MLST

340 indicate that extensive quality control checking at pre-retail stages should be implemented.

341 Furthermore, regular disinfecting of all equipment, as well as at working areas, must be applied.

342 Future efforts in strengthening food safety education and awareness programs would help

343   authorities to establish strategies that could potentially reduce the transmission of *Salmonella* and

344   other foodborne pathogens to downstream consumers.

345

346   **Acknowledgements**

356

357   **Disclosure Statement**

358   No competing financial interests exist.

359

360

361

362

363

364

365

366

367

368

369

370   **Figure Legends**

371   **Figure 1: Rate of resistance (%) to selected antimicrobials in *Salmonella* isolated from**

372   **various meat products in the Thai-Lao border area.**

373   Antibiotic abbreviations: amoxicillin-clavulanic acid (AMC); ampicillin (AMP);

374   chloramphenicol (C); ciprofloxacin (CIP); cefotaxime (CTX); nalidixic acid (NA); norfloxacin

375   (NOR); sulfamethoxazole-Trimethoprim (SXT); streptomycin (S); tetracycline (TE).

376

377   **Figure 2: Dendrogram generated using UPGMA algorithms based on MLST profiles,**

378   **including phenotypic characterization and the epidemiological meta data of *Salmonella***

379   **isolated in the Thai-Lao border area**.

380

381

382

383

384

385

386

387

388

389

390

391

392

393

394

395 **References**

396 Australian Embassy Thailand. (2011). The First Thai - Laos Friendship Bridge. *Available at*

397 *http://thailand.embassy.gov.au/bkok/FunRun_Bridge_History.html* (accessed 31 July

398 2017).

399 Basler C, Nguyen T-A, Anderson TC, Hancock T, Behravesh CB. 2016. Outbreaks of Human

400 *Salmonella* Infections Associated with Live Poultry, United States, 1990-2014. *Emerging*

401 *Infectious Diseases* 22(10):1705-1711. DOI: 10.3201/eid2210.150765.

402 Boonmar S, Morita Y, Pulsrikarn C, Chaichana P, Pornruagwong S, Chaunchom S, Sychanh T,

403 Khounsy T, Sisavath D, Yamamoto S, Sato H, Ishioka T, Noda M, Kozawa K, Kimura H.

404 2013. *Salmonella* prevalence in meat at retail markets in Pakse, Champasak Province,

405 Laos, and antimicrobial susceptibility of isolates. *Journal of Global Antimicrobial*

406 *Resistance* 1(3):157-161. DOI: 10.1016/j.jgar.2013.05.001.

407 Campioni F, Moratto Bergamini AM, Falcao JP. 2012. Genetic diversity, virulence genes and

408 antimicrobial resistance of *Salmonella* Enteritidis isolated from food and humans over a

409 24-year period in Brazil. *Food Microbiology* 32(2):254-264. DOI:

410 10.1016/j.fm.2012.06.008.

411   Clinical and Laboratory Standards Institute (CLSI). 2011. *Performance standards for*

412   *antimicrobial disc susceptibility test; Approved standard-Tenth Edition*. Wayne, PA:

413   Clinical and Laboratory Standards Institute.

414   Conlan JV, Vongxay K, Khamlome B, Gomez-Morales MA, Pozio E, Blacksell SD, Fenwick S,

415   Thompson RCA. 2014. Patterns and Risks of Trichinella Infection in Humans and Pigs in

416   Northern Laos. *PLOS Neglected Tropical Diseases* 8(7):e3034. DOI:

417   10.1371/journal.pntd.0003034.

418   Ferrari RG, Rosario DKA, Cunha-Neto A, Mano SB, Figueiredo EES, Conte-Junior CA. 2019.

419   Worldwide Epidemiology of *Salmonella* Serovars in Animal-Based Foods: a Meta-

420   analysis. *Applied and Environmental Microbiology* 85(14):e00591-00519. DOI:

421   10.1128/AEM.00591-19.

422   Foley SL, Lynne AM. 2008. Food animal-associated *Salmonella* challenges: pathogenicity and

423   antimicrobial resistance. *Journal of Animal Science* 86(14):E173-187. DOI:

424   10.2527/jas.2007-0447.

425   Gomes-Neves E, Antunes P, Tavares A, Themudo P, Cardoso MF, Gartner F, Costa JM, Peixe L.

426   2012. *Salmonella* cross-contamination in swine abattoirs in Portugal: Carcasses, meat and

427   meat handlers. *International Journal of Food Microbiology* 157(1):82-87. DOI:

428   10.1016/j.ijfoodmicro.2012.04.015.

429   Guardabassi L, Jensen LB, Kruse H. 2008. *Guide to Antimicrobial Use in Animals*. Oxford, UK:

430   Blackwell Publishing Ltd.

431   Harbottle H, White DG, McDermott PF, Walker RD, Zhao S. 2006. Comparison of multilocus

432   sequence typing, pulsed-field gel electrophoresis, and antimicrobial susceptibility typing

433   for characterization of *Salmonella enterica* serotype Newport isolates. *Journal of Clincal*

434   *Microbiology* 44(7):2449-2457. DOI: 10.1128/jcm.00019-06.

435 Hauser E, Hebner F, Tietze E, Helmuth R, Junker E, Prager R, Schroeter A, Rabsch W, Fruth A,

436   Malorny B. 2011. Diversity of *Salmonella enterica* serovar Derby isolated from pig, pork

437   and humans in Germany. *International Journal of Food Microbiology* 151(2):141-149.

438   DOI: 10.1016/j.ijfoodmicro.2011.08.020.

439 Heyndrickx M, Vandekerchove D, Herman L, Rollier I, Grijspeerdt K, De Zutter L. 2002.

440   Routes for *salmonella* contamination of poultry meat: epidemiological study from

441   hatchery to slaughterhouse. *Epidemiology and Infection* 129(2):253-265. DOI:

442   10.1017/s0950268802007380.

443 Jajere SM. 2019. A review of *Salmonella enterica* with particular focus on the pathogenicity and

444   virulence factors, host specificity and antimicrobial resistance including multidrug

445   resistance. *Veterinary World* 12(4):504-521. DOI: 10.14202/vetworld.2019.504-521.

446 Kotetishvili M, Stine OC, Kreger A, Morris JG, Sulakvelidze A. 2002. Multilocus Sequence

447   Typing for characterization of clinical and environmental *Salmonella* Strains. *Journal of*

448   *Clinical Microbiology* 40(5):1626. DOI: 10.1128/JCM.40.5.1626-1635.2002.

449 Kurtz JR, Goggins JA, McLachlan JB. 2017. *Salmonella* infection: Interplay between the

450   bacteria and host immune system. *Immunology Letters* 190:42-50. DOI:

451   10.1016/j.imlet.2017.07.006.

452 Liu WB, Liu B, Zhu XN, Yu SJ, Shi XM. 2011. Diversity of *Salmonella* isolates using

453   serotyping and multilocus sequence typing. *Food Microbiology* 289(6):1182-1189. DOI:

454   10.1016/j.fm.2011.04.001.

455   Lo Fo Wong DMA, Hald T, Van der Wolf P, Swanenburg M. 2002. Epidemiology and control

456   measures for *Salmonella* in pigs and pork. *Livestock Production Science* 76:215-222.

457   DOI: 10.1016/S0301-6226(02)00121-5.

458   Mainali C, Gensler G, Mcfall M, King R, Irwin R, Senthilselvan A. 2009. Evaluation of

459   Associations between Feed Withdrawal and Other Management Factors with *Salmonella*

460   Contamination of Broiler Chickens at Slaughter in Alberta. *Journal of Food Protection*

461   72(10):2202–2207.

462   Mughini-Gras L, Enserink R, Friesema I, Heck M, van Duynhoven Y, van Pelt W. 2014. Risk

463   factors for human salmonellosis originating from pigs, cattle, broiler chickens and egg

464   laying hens: a combined case-control and source attribution analysis. *PLoS One*

465   9(2):e87933-e87933. DOI: 10.1371/journal.pone.0087933.

466   Napasirth P, Napasirth V. 2018. Current situation and future prospects for beef production in Lao

467   People's Democratic Republic - A review. *Asian-Australasian Journal of Animal*

468   *Sciences* 31(7):961-967. DOI: 10.5713/ajas.18.0206.

469   Okello AL, Tiemann TT, Inthavong P, Khamlome B, Phengvilaysouk A, Keonouchanh S,

470   Keokhamphet C, Somoulay V, Blaszak K, Blacksell SD, Okello WO, Allen J. 2017.

471   Integrating market chain assessments with zoonoses risk analysis in two cross-border pig

472   value chains in Lao PDR. *Asian-Australasian Journal of Animal Sciences* 30(11):1651-

473   1659. DOI: 10.5713/ajas.16.0887

474   Padungtod P, Kaneene JB. 2006. *Salmonella* in food animals and humans in northern Thailand.

475   *International Journal of Food Microbiology* 108(3):346-354. DOI:

476   10.1016/j.ijfoodmicro.2005.11.020.

477   Patchanee P, Eiamsam-Ang T, Vanaseang J, Boonkhot P, Tadee P. 2017. Determination of

478   regional relationships among *Salmonella* spp. isolated from retail pork circulating in the

479   Chiang Mai municipality area using a WGS data approach. *International Journal of Food*

480   *Microbiology* 254:18-24. DOI: 10.1016/j.ijfoodmicro.2017.05.006.

481   Patchanee P, Tansiricharoenkul K, Buawiratlert T, Wiratsudakul A, Angchokchatchawal K,

482   Yamsakul P, Yano T, Boonkhot P, Rojanasatien S, Tadee P. 2016. *Salmonella* in pork

483   retail outlets and dissemination of its pulsotypes through pig production chain in Chiang

484   Mai and surrounding areas, Thailand. *Preventive Veterinary Medicine* 130:99-105. DOI:

485   10.1016/j.prevetmed.2016.06.013.

486   Phongaran D, Khang-Air S, Angkititrakul S. 2019. Molecular epidemiology and antimicrobial

487   resistance of *Salmonella* isolates from broilers and pigs in Thailand. *Veterinary World*

488   12(8):1311-1318. DOI: 10.14202/vetworld.2019.1311-1318.

489   Popoff MY, Bockemühl J, McWhorter-Murlin A. 1993. Supplement 1992 (no. 36) to the

490   Kauffmann-White scheme. *Research in Microbiology* 144(6):495-498. DOI:

491   doi.org/10.1016/0923-2508(93)90058-A.

492   Pulsrikarn C, Chaichana P, Pornruangwong S, Morita Y, Yamamoto S, Boonmar S. 2012.

493   Serotype, Antimicrobial Susceptibility, and Genotype of *Salmonella* Isolates from Swine

494   and Pork in Sa Kaew Province, Thailand. *The Thai Journal of Veterinary Medicine*

495   42(1):21-28.

496   Rostagno MH, Callaway TR. 2012. Pre-harvest risk factors for *Salmonella enterica* in pork

497   production. *Food Research International* 45(2):634-640. DOI:

498   10.1016/j.foodres.2011.04.041.

499   Shang K, Wei B, Kang M. 2018. Distribution and dissemination of antimicrobial-resistant

500   *Salmonella* in broiler farms with or without enrofloxacin use. *BMC Veterinary Research*

501   14(1):257. DOI: 10.1186/s12917-018-1590-1.

502   Sinwat N, Angkittitrakul S, Coulson KF, Pilapil FM, Meunsene D, Chuanchuen R. 2016. High

503   prevalence and molecular characteristics of multidrug-resistant *Salmonella* in pigs, pork

504   and humans in Thailand and Laos provinces. *Journal of Medical Microbiology*

505   65(10):1182-1193. DOI: 10.1099/jmm.0.000339.

506   Sirichote P, Bangtrakulnonth A, Tianmanee K, Unahalekhaka A, Oulai A, Chittaphithakchai P,

507   Kheowrod W, Hendriksen RS. 2010. Serotypes and Antimicrobial Resistance of

508   *Salmonella enterica* ssp in Central Thailand, 2001-2006. *The Southeast Asian journal of*

509   *tropical medicine and public health* 41(6):1405-1415.

510   Trongjit S, Angkititrakul S, Tuttle R, Poungseree J, Padungtod P, Chuanchuen R. 2017.

511   Prevalence and antimicrobial resistance in *Salmonella enterica* isolated from broiler

512   chickens, pigs and meat products in the Thailand-Cambodia border provinces: AMR in

513   *Salmonella enterica*. *Microbiology and Immunology* 61. DOI: 10.1111/1348-0421.12462.

514   University of Warwick. EnteroBase: *Salmonella enterica* MLST Database. Available at

515   http://enterobase.warwick.ac.uk/species/senterica/allele_st_search (accessed 22 June

516   2020).

517   University of Zaragoza. Win Epi: Sample size: Estimate Percentage. Available at

518   http://www.winepi.net/uk/index.htm (accessed on 21 December 2018).

519   Van Boxstael S, Dierick K, Van Huffel X, Uyttendaele M, Berkvens D, Herman L, Bertrand S,

520   Wildemauwe C, Catry B, Butaye P, Imberechts H. 2012. Comparison of antimicrobial

521   resistance patterns and phage types of *Salmonella* Typhimurium isolated from pigs, pork

522   and humans in Belgium between 2001 and 2006. *Food Research International* 45(2):913-

523   918. DOI: 10.1016/j.foodres.2011.05.025.

524   Wilson RT. 2007. Status and prospects for livestock production in the Lao People's Democratic

525   Republic. *Tropical Animal Health and Production* 39(6):443-452. DOI: 10.1007/s11250-

526   007-9048-7.

527   Yang Y, Ashworth AJ, Willett C, Cook K, Upadhyay A, Owens PR, Ricke SC, DeBruyn JM,

528   Moore Jr. PA. 2019. Review of Antibiotic Resistance, Ecology, Dissemination, and

529   Mitigation in U.S. Broiler Poultry Systems. *Frontiers in Microbiology* 10:e2639. DOI:

530   10.3389/fmicb.2019.02639.

531   Zhou Z, Alikhan NF, Mohamed K, Fan Y, Agama Study Group, Achtman, M. 2020. The

532   EnteroBase user's guide, with case studies on *Salmonella* transmissions, *Yersinia*

533   *pestis* phylogeny, and *Escherichia* core genomic diversity. *Genome research 30*(1):138–

534   152. DOI: 10.1101/gr.251678.119.

Figure 1

Rate of resistance (%) to selected antimicrobials in *Salmonella* isolated from various meat products 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).
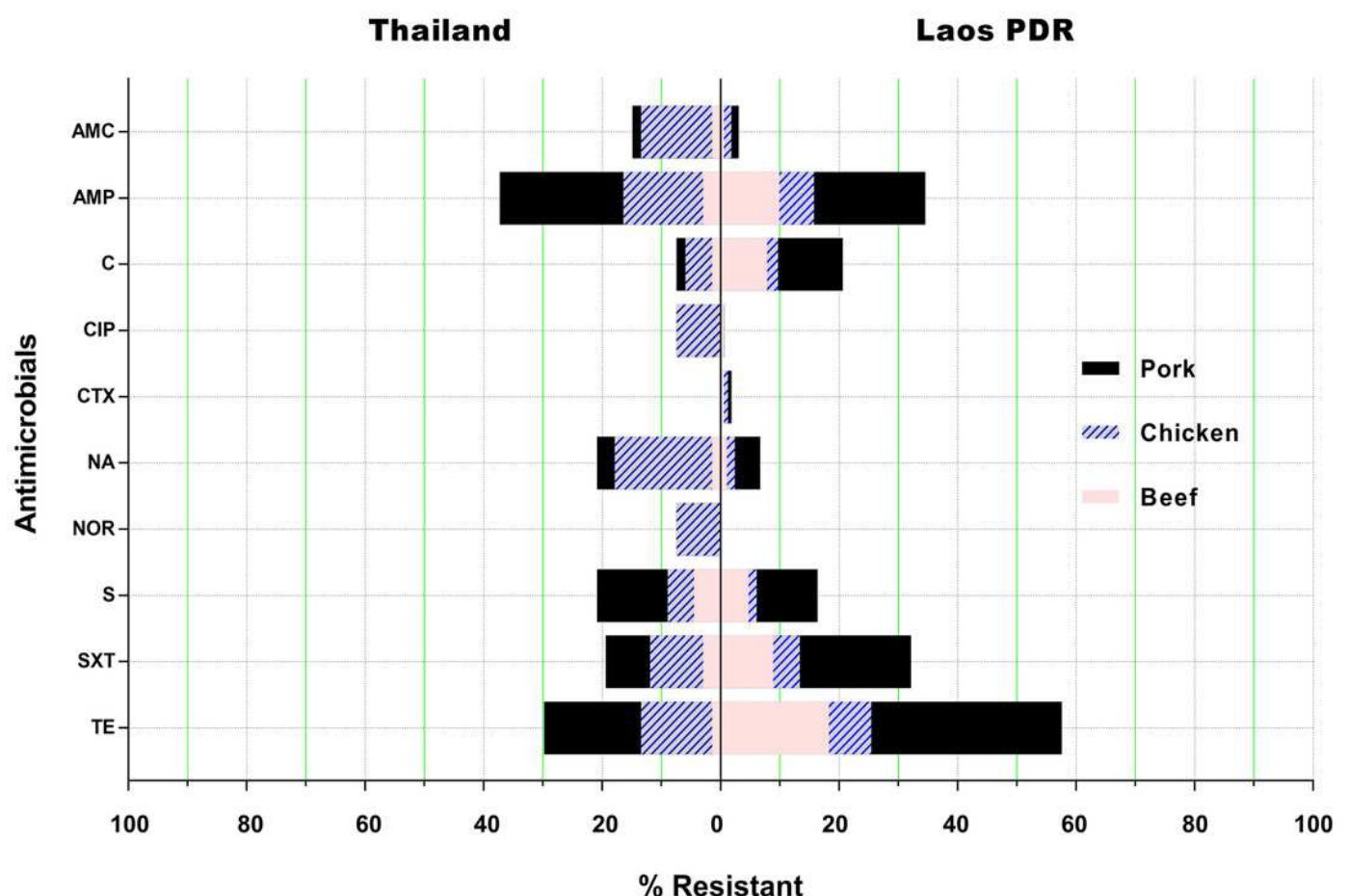
Figure 2

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

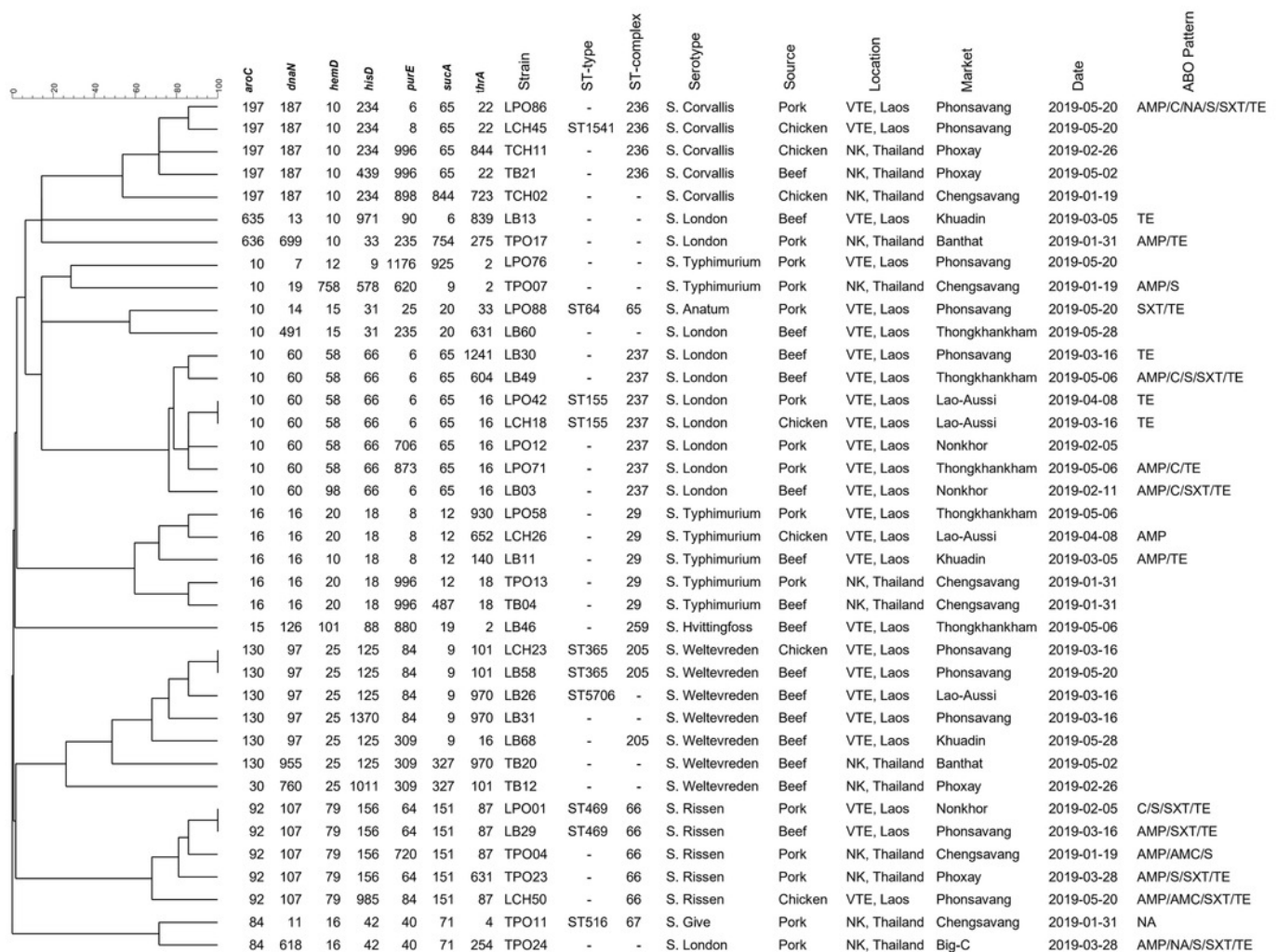1 **Table 1** Distribution of prevalence and a 95% confidence interval of *Salmonella* isolated from

2 various meat types in the Thai-Lao border area.

3

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

4

5 Use of fisher's exact analysis and difference of superscript (A, B) indicate significant differences

6 ($p < 0.05$) of prevalence detected among meat types. Difference of superscript (a, b) indicates

7 significant differences ($p < 0.05$) of prevalence detected among all locations.

8

Table 2(on next page)

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

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

2 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

Serotype							Total	%
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

3

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.

1   **Table 3** Variation of housekeeping genes loci of un-identified Sequence Type (ST) *Salmonella*

2   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

3

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

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