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Research article

Salmonella linkaged between good agricultural practice certified broiler farms and poultry slaughterhouses in an intensive farming area in upper northern part of Thailand

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Abstract

Non-typhoidal *Salmonella* (NTS) is one of the most contagious foodborne infections and continues to be a major global health threat in both human and veterinary medicine. Poultry meat was one of the important sources of NTS spreading and tended to be highly resistant to antibiotics. The objective of this study was to identify the prevalence, serotypes, and antimicrobial resistance patterns of *Salmonella* that were present in broiler farms and poultry slaughterhouses in an intensive farming region in the upper northern part of Thailand from August to October 2019. Fifty samples of boot swabs were collected from 50 farms,50 cecal samples, and 250 neck skin samples from slaughterhouses. *Salmonella* was identified by culture method and serum-agglutination and antimicrobial susceptibility testing was performed using the automated VITEK-2 compact system. This study's total prevalence of *Salmonella* was 53.71% (n=188/350). We found that 53% (159/300) of cecal and neck skin samples were collected from slaughterhouses and 58% (29/50) of boot swabs collected from farms were positive for NTS. Twenty-four serotypes of NTS were identified, the most encountered was *S*. Kentucky. Most of the serotypes were found in neck skin samples, followed by cecal and fecal samples. The antimicrobial-resistant patterns showed that all the strains were non-susceptible to amikacin, cefalexin, cephalothin, and gentamicin and were susceptible to imipenem, neomycin, and nitrofurantoin. The incidence of NTS in this study indicates contamination in the farming and slaughtering processes. As a result, it is critical to evaluate both antimicrobial usage and hygienic standards for broiler production.

Keywords: Antimicrobial resistance and upper northern Thailand, Broiler, Prevalence, Salmonella spp.

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INTRODUCTION

Non-typhoidal *Salmonella* (NTS) continues to be one of the most contagious foodborne infections, posing serious problems for both human and veterinary medicine globally. (Ryan et al., 2017) NTS is an etiological agent of diarrhea and systemic infections. According to statistics from the United States, *Salmonella* caused 122 outbreaks, 3,061 illnesses, and 478 hospitalizations compared to other foodborne pathogens in its surveillance region. (CDC, 2019). Salmonellosis, a serious infectious disease that affects both humans and animals, was caused by *Salmonella. Salmonella* contamination in animal products is always brought on by an illness in food animals. (WOAH, 2018). NTS may enter an animal farm through feed, water, pasture, waste, or contact with another animal. *Salmonella* can be transmitted from farm animals to humans by consuming animal products, among other things. (Kemal, 2014) Due to the fact that poultry meat was a significant source of *Salmonella* spp. transmission from poultry to humans (Chotinun et al., 2014). *Salmonella* spp. prevalence in broiler production in Thailand between 2009 and 2011 revealed that broiler farms have the highest prevalence, followed by parent stock farms and hatcheries, which have prevalences between 21.63% and 32.88%, 6.75% to 11.56%, and 2.93% to 7.35%. *Salmonella* spp. can therefore be identified in all stages of the broiler production chain, with farm level presumably having the largest means and range of occurrence (Rugpudsa et al., 2013). The Agricultural Standards Committee of Thailand produced a standard known as Good Agricultural Practices (GAP) for broiler farms. This guideline is appropriate management for broiler farms and a good hygienic practice that can reduce *Salmonella* contamination in broilers. This standard aims to provide safe, wholesome broilers. The Department of Livestock Development developed a *Salmonella* surveillance program as well for tracking risk factors and *Salmonella* prevalence in broiler farms with Good Agricultural Practice certification. The upper northern region of Thailand has the second-highest frequency of *Salmonella* spp. in all of Thailand, according to a prior study conducted on Good Agricultural Practice-certified broiler farms in Thailand in 2014 (Suddee et al., 2014).

Furthermore, sanitation in the slaughtering process should be considered because a salmonellosis case in a human was reported as a result of contamination in the slaughterhouse (Mead et al., 1999). A study in Malaysia discovered *Salmonella* isolates in both a wet market and a small processing plant. (2017) (Nidaullah et al.)

Antibiotic use in the food animal business has become essential due to increased antibiotic resistance of *Salmonella* spp. (Tan et al., 2022). Inappropriate antibiotic use in animals resulted in bacterial resistance, which posed a concern to human health via animal food items. (Angulo et al., 2004). *Salmonella* spp. obtained from poultry is often resistant to fluoroquinolones (Rozman et al., 2018). A study in 2007 found that antimicrobials are routinely used on broiler farms in Thailand. The most commonly used drugs were enrofloxacin, amoxicillin, doxycycline, colistin, and roxithromycin. (Na Lampang et al., 2007).

Salmonella contamination was discovered at various levels of the poultry production chain in prior research. As a result, the purpose of this study was to investigate *Salmonella* prevalence, serotypes, and antimicrobial-resistant patterns of NTS isolated from broiler farms and poultry slaughterhouses in the upper northern part of Thailand in order to understand the source of contamination at both levels, which can be useful in implementing effective *Salmonella* control measures in the poultry production chain.

MATERIALS AND METHODS

Sample size calculation and Study site

The sample size was determined by conducting a calculation using the Epi Info v5.5.10 program, considering a population of 311 GAP certified broiler farms located in Chiang Mai, Lamphun, and Lampang. The prevalence rate used in the calculation was 79.17%, representing the proportion of farms with a specific characteristic. The desired confidence level was set at 95%, indicating the level of confidence required for the estimated prevalence to fall within the determined range. An accepted error of 10% was also considered, representing the maximum allowable difference between the estimated prevalence and the true prevalence. As a result of this calculation, a sample size of 50 GAP farms was obtained. These farms were randomly selected based on the determined sample size for inclusion in the study. (DLD, 2018)

Sample collection

All samples were obtained between August and October of 2019. Fifty fecal samples were gathered from 50 GAP-certified broiler farms using the boot swab technique, which involved walking around the house on the beds to collect feces, as an optional step not included in the sample from the calculation, 31 samples of chick paper from 31 farms that still possessed them were collected. The same broiler flock was then trailed from these farms to the slaughterhouses. Ceca and neck skins were taken from broiler carcasses at the slaughterhouse. Ten ceca from ten carcasses were pooled for one sample per farm (50 cecal samples from 50 farms) (DLD, 2016) and three neck skins (10 g per piece) were pooled from three carcasses for one sample, with five samples required per farm (250 neck skin samples from 50 farms) (EU, 2011). Prior to the isolation of *Salmonella* spp., all collected samples were frozen at 4^{o}C and sent to the bacteriology and mycology laboratory, diagnostic section, Veterinary Research and Development Center (Upper Northern Region).

Salmonella isolation and identification

Salmonella spp. were isolated and identified from fecal, cecal, neck skin, (350 samples) and chick paper samples (31 samples) in accordance with ISO 6579-1: 2017 (ISO, 2017). All samples (25 g) were pre-enriched in Buffered Peptone Water (BPW; Oxoid, Hampshire, UK) at a weight/vol ratio of 1:10 and incubated at 37 ± 1°C for 18 ± 2 h. Pre-enriched samples were incubated at 41.5 ± 1°C for 24 to 48 hours in Semi-Solid Modification Rappaport Vassiliadis (MSRV; Merck, New Jersey, USA). The cultures from the positive MSRV plate were inoculated onto Xylose Lysine Deoxycholate Agar (XLD; Oxoid, Hampshire, UK) and Brilliant Green Agar (BGA; Oxoid, Hampshire, UK) and incubated at 37 ± 1°C for 24 ± 3 h. After incubation, five typical *Salmonella* colonies were confirmed by a biochemical test set, including triple sugar iron agar (TSI; Oxoid, Hampshire, UK), urea agar base (Urea; Merck, New Jersey, USA), and motility indole lysine medium (MIL; BD, New Jersey, USA). Only one *Salmonella* strain from each sample was chosen for further investigation.

Serotyping and Antimicrobial susceptibility testing

Ninety-six *Salmonella* strains from farms and slaughterhouses and six *Salmonella* strains from chick paper were serotyped by serum-agglutination according to the White-Kauffmann-Le Minor scheme (Grimont and Weill, 2007). Antimicrobial susceptibility testing was performed in 50 *Salmonella* strains with the automated VITEK 2 compact system based on MIC technique determination using the VITEK 2 gram negative susceptibility cards (AST-GN97), which contained amikacin (AN), cefalexin (CN), cefalotin (CF), gentamicin (GM), ampicillin (AM), pradofloxacin (PRA), enrofloxacin (ENR), marbofloxacin (MRB), doxycycline (DO), amoxicillin-clavulanic acid (AMC), tetracycline (TE), cefpodoxime (CPD), cefovecin (CFO), ceftiofur (CFT), chloramphenicol (C), sulfamethoxazole Trimethoprim (SXT), imipenem (IPM), neomycin (N) and nitrofurantoin (FT) .The minimum inhibitory concentration result of each *Salmonella* isolate was visualized using binary heatmap analysis, which represents the susceptible or non-susceptible result (the intermediate and resistant results were interpreted as the non-susceptible result in this study). The dendrogram of the resistance patterns was constructed using the unweighted pair group method with arithmetic mean (UPGMA) algorithms according to the Jaccard correlation by the BioNumerics version 7.6 program.

RESULTS

Salmonella prevalence

A total of 350 samples were gathered from both the farm and the slaughterhouse, and 188 of them tested positive for *Salmonella***.** This results in a prevalence rate of 53**.**71% (n=188/350, 95% CI= 48.33-59.03). 50 fecal samples were taken on the broiler farm, and 29 of them tested positive for *Salmonella*. This represents a 58% prevalence rate. (n=29/50, 95% CI= 43.21-71.81). Two types of samples were collected at the poultry slaughterhouse level: neck skin and cecum. 250 neck skin samples and 50 cecal samples were collected. *Salmonella* was found in 159 samples from both. This leads to a 53% prevalence rate (n=159/300, 95% CI= 47.18-58.76) As a result, the prevalence of *Salmonella* is higher on the farm than in the slaughterhouse as shown in Table 1**.**

Table 1 *Salmonella* prevalence isolated from farm and slaughterhouse during August to October 2019 in Upper Northern part of Thailand

Sample type		**No. of samples**	**No. of Positive samples**	**Prevalence**	**95% CI**
Farm	Feces	50	29	58	43.21-71.81
Slaughterhouse	Neck skins	250	147	53	47.18-58.76
	Cecum	50	12		
Total		350	188	53.71	48.33-59.03

Salmonella sero-distribution

Salmonella serotypes were found in 24 serotypes, with *S.* Kentucky being the most common, followed by *S.* Livingstone, *S.* Albany, and *S.* Uganda, as indicated in Table 2. The table shows the distribution of different *Salmonella* serotypes from farms and slaughterhouses, as well as an overview of all positive samples from both sources and the distribution pattern of *Salmonella* from various sources is depicted in Figure 1**.** The figure shows the distribution of serotypes found mostly in the neck skin, followed by the cecum, feces, and chick papers**.**

Table 2 Prevalence of *Salmonella* serotypes isolated from farm and slaughterhouse samples

***Salmonella* serovars**	**Farm (N=23) n (%)**	**Slaughterhouse(N=73) n (%)**	**Total (N=96) n (%)**
S. Adabraka		1 (1.37)	1 (1.04)
S. Agona	3 (13.04)	1 (1.37)	4 (4.16)
S. Albany	6 (26.08)	5 (6.85)	11 (11.46)
S. Altona		2 (2.74)	2 (2.08)
S. Brunei		1 (1.37)	1 (1.04)
S. Corvallis		4 (4.11)	4 (4.16)
S. Dabou		1 (1.37)	1 (1.04)
S. Enterica		1 (1.37)	1 (1.04)
S. Hvittingfoss	1(4.34)	2 (2.74)	3 (3.12)
S. Hadar		1 (1.37)	1 (1.04)
S. Hartford		1 (1.37)	1 (1.04)
S. Jerusalem		1 (1.37)	1 (1.04)
S. Kentucky	2 (8.69)	15 (20.54)	17 (17.71)
S. Livingstone	1 (4.34)	13 (17.81)	14 (14.58)
S. Mbandaka	1 (4.34)		1 (1.04)
S. Muenster	1 (4.34)	2 (2.74)	3 (3.12)
S. Okerara	1 (4.34)		1 (1.04)
S. Orion		2 (2.74)	2 (2.08)
S. Saintpaul	1 (4.34)	5 (6.85)	6 (6.25)
S. Shleissheim		1 (1.37)	1 (1.04)
S. Singapore		3 (4.10)	3 (3.12)
S. Stanley	1 (4.34)		1 (1.04)
S. Uganda		11 (15.06)	11 (11.46)
S. Weltevreden	5 (21.73)		5 (5.21)

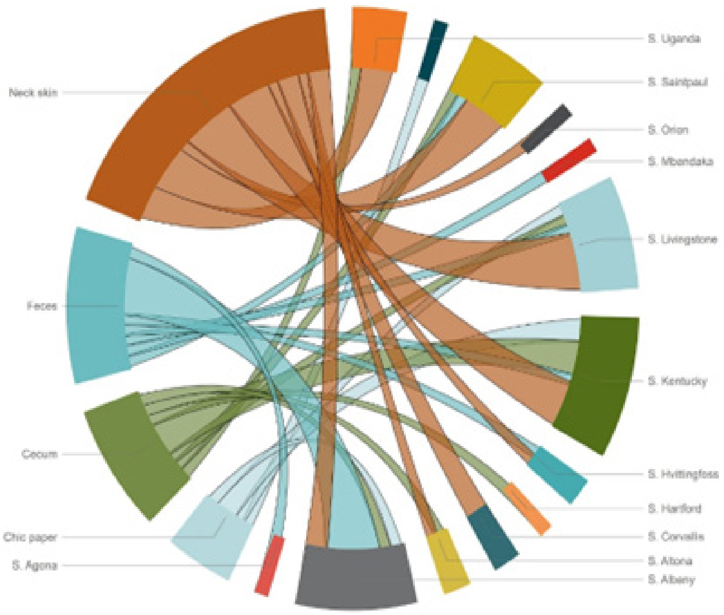

Figure 1 *Salmonella* serotype-distribution in feces, cecum, neck skin and chic paper. This figure shows the distribution of *Salmonella* serotypes found mostly in the neck skin, followed by the cecum, feces, and chick paper. All sample sources contained *S*. Kentucky. *S*. Mbandaka was only discovered in feces. *S*. Orion was only detected in the neck skin, and *S*. Hartford was only found in the cecum.

Antimicrobial susceptibility testing

Fifty *Salmonella* isolates were selected for the purpose of investigating their antimicrobial-resistant patterns. The analysis revealed that all the strains exhibited resistance to amikacin (100%), cefalexin (100%), cephalothin (100%), and gentamicin (100%). However, it was observed that all *Salmonella* strains tested were susceptible to imipenem, neomycin, and nitrofurantoin. *Salmonella* antimicrobial resistance patterns were analyzed by Jaccard correlation, represented by a phylogenetic tree, demonstrating the correlation of antimicrobial resistance patterns between each serotype sample**.** The antimicrobial resistance patterns associated with each serotype show variations**.** Most serotypes in the same clade exhibit the same resistance pattern as shown in Figure 2**.**

Figure 2 *Salmonella* antimicrobial resistance patterns analyzed by Jaccard correlation. This figure shows *Salmonella* antimicrobial resistance patterns as represented by a phylogenetic tree, demonstrating the correlation of antimicrobial resistance patterns between each serotype sample. The black square in the figure represents a drug resistance pattern, whereas the white square represents a drug sensitivity pattern. As illustrated in the figure, most serotypes in the same clade exhibit the same resistance pattern.

DISCUSSION

Salmonella contamination in poultry production can be detected through the whole production chain, specifically in the farming and slaughtering processes. To determine the precise source of contamination in both processes, which will lead to the development of effective *Salmonella* control policies at each stage of the production chain. *Salmonella* prevalence in GAP-certified broiler farms was 58% in this study, down from 79.17% in the study of Suddee et al. in 2014 in the same area. This could be due to a strategy implemented by the Department of Livestock Development's national *Salmonella* control and surveillance program, which has been updated for use on GAP-certified broiler farms since 2015. *Salmonella* management measures include an intensive biosecurity approach, bedding disinfection, visiting restriction, farmer personal hygiene, and pest control. The results of this study indicated that proper biosecurity management is one of the most significant factors in avoiding or minimizing *Salmonella* infection on farms. In this study, *S*. Albany, *S*. Agona, *S*. Mbandaka, and *S*. Weltevreden from poultry farms resembled studies from Chiang Mai and Lamphun. (Na Lampang et al., 2014), and *S*. Weltevreden is regarded as the common serotype in broilers raised in Thailand. Padungtod et al. (2006) This discovery suggested that all those serotypes had already been circulating in this farming region.

According to the study of Phongaran et al. from 2019 and this study, *S.* Kentucky was the most prevalent serotype discovered in slaughterhouses. According to the study of Salehi et al., 2016, *S.* Kentucky is also the most common serotype in chicken processing plants in the United States and Europe. Many serotypes were discovered at the slaughterhouse level in the broiler's neck skin, which represented the hygiene of the facility, including the sanitation of the environment and the equipment utilized during the slaughtering process. The size of the slaughterhouse may affect the level of contamination. In comparison to small-scale slaughterhouses, large slaughterhouses have more customers who come from a variety of diverse sources, which is likely to result in an increase in the contamination rate. To reduce the contamination of chicken meat, which can expose customers to that risk, slaughtering procedures and good hygienic management must be strictly observed in slaughterhouses. The significant serotypes *S.* Typhimurium and *S.* Enteritidis that were known to cause severe gastroenteritis in humans were not identified in this study. The majority of the serotypes identified in this investigation were common serotypes that were previously prevalent in environments on both farms and slaughterhouses.

There are differences in the correlations between antimicrobial resistance patterns in each serotype sample**.** Even within the same serotype, the pattern of antibiotic resistance varied; this result may indicate that there are many routes of transmission throughout the production chain, from hatchery to slaughterhouse**.** Most serotypes within the same clade have the same resistance pattern, indicating a shared source of contamination**.** A further study of the contamination factors in the production chain is required**.**

The selected *Salmonella* isolates were resistant to amikacin and gentamicin, similar to the study of Lauteri et al., 2022. Gentamicin is primarily used to treat bacterial infections in poultry. A study in Nigeria discovered that gentamicin use is progressing without a withdrawal period, resulting in a high incidence of drug residues in broiler meat. (Onyeanu et al., 2020). The improper use of antibiotics in livestock can lead to antimicrobial resistance in humans via food consumption, particularly chicken meat, which is the most commonly consumed high-protein diet. As a result, antimicrobial resistance in *Salmonella* can be found all over the world (Zdragas et al., 2012). Antimicrobial resistance should be a major worry for all of the reasons stated above.

CONCLUSION

Hygienic measures in the broiler production chain, including antimicrobial usage, should be strictly considered at both chicken farms and slaughterhouses. *Salmonella* contamination in the broiler production chain should be confirmed using molecular epidemiology.All selected serotypes have already been forwarded to the University of Oxford in England for whole genome sequence analysis. A global epidemiological analysis of *Salmonella* must be warranted.

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AUTHOR CONTRIBUTIONS

Conceptualization and methodology designation: TB, PP
Sample collection: TB, SK
Performed the laboratory analysis: TB, NK
Analyzed the data: TB, TE, PP
Contributed reagents/materials/analysis tools: TB, NK, WS
Wrote the paper (review and editing): TB, PT, SC, PC, SK, BP, PP, NK.

CONFLICT OF INTEREST

All authors have no conflicts of interest to declare.

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