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Research article

Prevalence and antimicrobial resistance of *Salmonella* isolated from backyard pigs in Chiang Mai, Thailand

Chatchawan Anuchatkitcharoen[1], Sureerat Numee[2], Jeff Bender[3], Nattakarn Awaiwanont[2,4] and Montira Intanon[1,4*]

[1]Department of Veterinary Bioscience and Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand
[2] Department of Food Animal Clinics, Faculty of Veterinary Medicine, Chiang Mai University, 50100 Chiang Mai, Thailand
[3] School of Public Health, University of Minnesota, Saint Paul, Minneapolis, USA
[4] Veterinary Public Health and Food Safety Centre for Asia Pacific, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand

Abstract

Salmonella is a zoonotic, global public health challenge. In Southeast Asia, backyard pigs are common and there is limited information about the prevalence of *Salmonella* and their risks to humans. This study was designed to determine prevalence and the antimicrobial resistance patterns of *Salmonella* in backyard pigs in Chiang Mai, Thailand. Ninety-three pooled fecal samples were collected from backyard pigs in three regions of Chiang Mai, Thailand during November 2016 – March 2017 for *Salmonella* isolation and antimicrobial susceptibility testing. The pooled prevalence of *Salmonella* in backyard pigs was 21.5%. Nine *Salmonella* serovars were identified including *Salmonella* I 4,5,12:i:- (31.0%), *Salmonella* Weltevreden (17.2%), *Salmonella* Rissen (13.8%), *Salmonella* Bovismorbificans (10.3%) and *Salmonella* Stanley (10.3%). The *Salmonella* isolates were commonly resistant to streptomycin followed by tetracycline, ampicillin and sulfamethoxazole/trimethoprim. No isolates were resistant to cefoxitin, nalidixic acid or imipenem. Sixteen different multi-resistant pattern were observed among isolates. The most frequent multi-resistant pattern was AMP-TET-SXT-S. Backyard pigs were harboring *Salmonella* and can serve as reservoirs transmitting infections to humans and other backyard animals. A variety of different serovars were isolated with a broad range of resistance profiles to different classes of antimicrobial agents. This provides evidence of the importance of educating owners and their families about the potential public health risk.

Keywords: Antimicrobial resistance, Backyard pigs, *Salmonella*

***Corresponding author:** Montira Intanon, Department of Veterinary Bioscience and Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand. Tel: + 66 939814106 Email: montira.intanon@cmu.ac.th.

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INTRODUCTION

Salmonella is a common foodborne pathogen affecting people throughout the world (Galanis et al., 2006). Most human salmonellosis is caused by *Salmonella* contamination of food; however, direct contact with animals can also serve as a source of infection. Food animals such as poultry, pigs and cattle are often identified as the main sources of the infections. (Bangtrakulnonth et al., 2004; Hale et al., 2012; Manning et al., 2015). At present, more than 2,600 serovars, *Salmonella* Enteritidis and *Salmonella* Typhimurium are the two most common serovars that were isolated and responsible for animals and human salmonellosis. However, predominant serovars can varies between different geographic locations (Jajere, 2019; Jain et al., 2020). Pigs are the reservoirs of *Salmonella*. The predominant *Salmonella* serovars from pigs in Thailand are *S*. Rissen and *S*. Typhimurium (Trongjit et al., 2017; Phongaran et al., 2019). Also, there is growing concern about multidrug-resistant (MDR) *Salmonella*, especially the effectiveness of important antimicrobial agents , such as fluoroquinolones and extended-spectrum cephalosporins, which are the drug of choices used for treatment of invasive salmonellosis in the pediatric patient (Chiu et al., 2002; Crump and Mintz, 2010).

Chiang Mai has the highest number of backyard pigs in Thailand with 84,587 pigs reared by 12,208 households (Department of Livestock Development, 2014). Backyard pig production system is typically small scale with poor biosecurity management practice and allows for close contact between the backyard animals, farmers, their families, and wildlife animals (Alegria et al., 2017a). In rural areas, backyard pigs are a significant source of socio-economic income in communities. However, they are not usually included in the routine government monitoring programs for *Salmonella*. *Salmonella* from backyard pigs can potentially be a significant zoonotic risk to human and also lead to morbidity in animals and economic loss (Alegria et al., 2017b). Currently, the data regarding *Salmonella* in backyard pigs in Chiang Mai, Thailand is limited. Our study aim was to determine the prevalence of *Salmonella*, common serovars and antimicrobial resistance profile of backyard pigs in Chiang Mai, Thailand.

MATERIALS and METHODS

Study design and sample size

A cross-sectional study was conducted in backyard pigs in Chiang Mai province, Thailand during November 2016 – March 2017. Multi-stage cluster sampling was applied based on geographical locations. Briefly, Chiang Mai divided into 3 zones; north, central and south. Consequently, district, sub-district, village and household level were randomly selected from each zone. The study sites were shown in Figure 1. A total of 93 households from 15 districts participated in this study. The protocol for this study was approved by the Animal Care and Use Committee of the Faculty of Veterinary Medicine, Chiang Mai University, Thailand (No. S4/2559, date 18 March 2016).

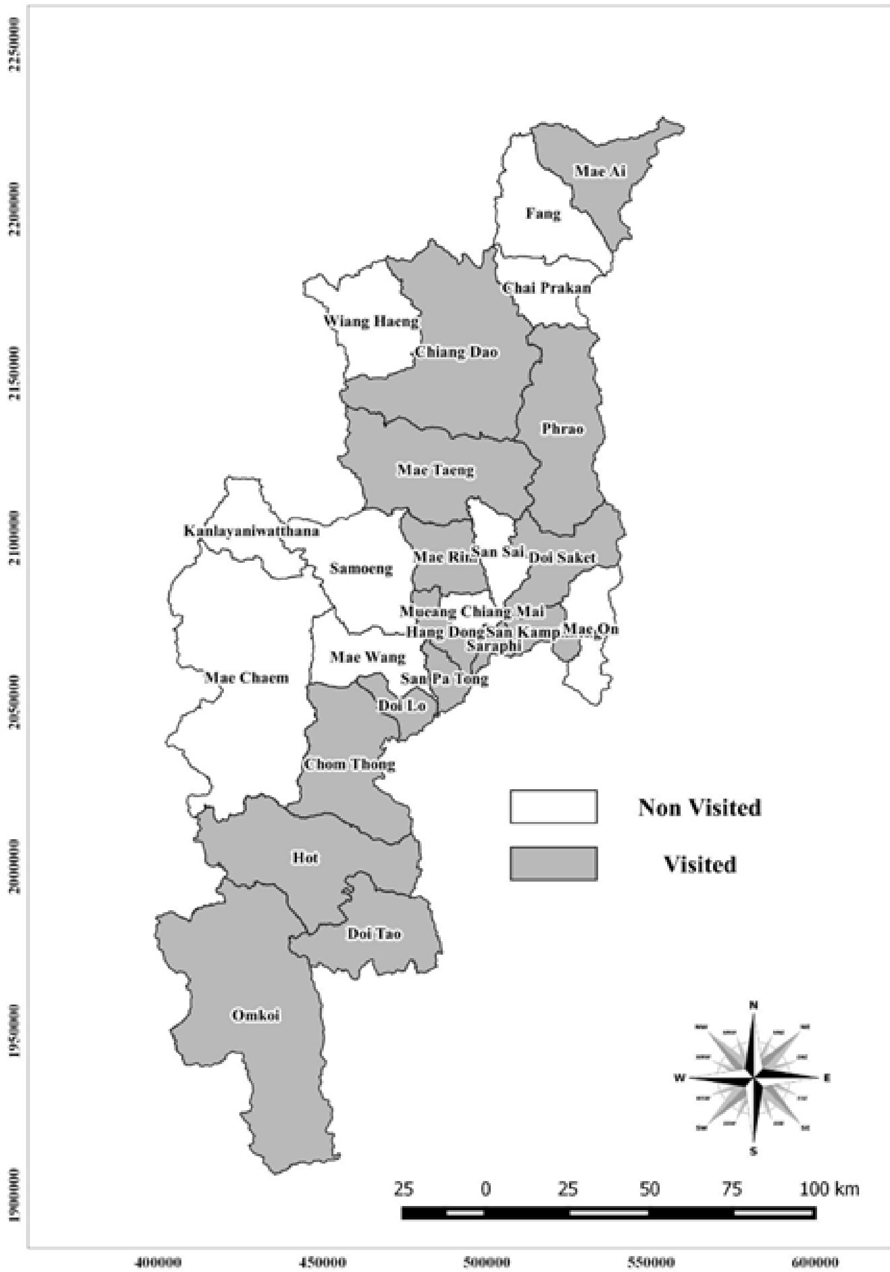

Figure 1 Geographical location of districts in Chiang Mai, Thailand. Samples were collected from the districts shown in gray.

Sample collection

Ninety-three fecal samples were collected from finishing pigs. Only if finishing pigs were not available, samples were collected from sows and piglets instead. The feces from 4-6 pigs per household were collected as a pooled sample. Samples were collected from the center of freshly excreted feces using a sterile plastic spoon. Spoons were placed in a sterile plastic bag and stored at 4-8°C before culturing. Cultures were started within 24 hours of collection.

Salmonella isolation

Salmonella isolation was conducted following (ISO 6579:2002/Amd 1:2007 Annex D) standard methodology. In brief, 25 g of a fecal sample was mixed with 225 ml buffer peptone water (BPW) (Merck®, Germany) using stomacher machine for 1 min and incubated at 37°C for 24 hours. Then, 0.1 ml of the incubated BPW was transferred to Modified Semisolid Rappaport-Vassilliadis (MSRV) medium (Merck®, Germany) and incubated at 41.5°C for 24-48 hours. Then, sub-cultured on two selective enrichment medias; Brilliant-green Phenol-red Lactose Sucrose agar (BPLS) (Merck®, Germany) and Xylose Lysine Deoxycholate agar (XLD) (Merck®, Germany) and incubated at 37°C for 24 hours. From each sample, 2-3 suspect *Salmonella* colonies were randomly selected and kept in 20% glycerol at -80°C for further study.

Species confirmation and Salmonella serovars identification

Presumptive *Salmonella* isolates were confirmed species using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) (Bruker Daltonics, Germany). Confirmed *Salmonella* isolates were serotyped according to the Kauffman-White serotyping scheme using *Salmonella* antisera (S&A Reagent Laboratory LMT, Bangkok, Thailand) (Bangtrakulnonth et al., 2004).

Antimicrobial resistance

The *Salmonella* isolates were tested for antimicrobial susceptibility using the disk diffusion method according to Clinical and Laboratory Standard Institute document (CLSI, 2012) against 11 antimicrobial agents including ampicillin 10 µg (AMP), amoxicillin/clavulanic acid 20/10 µg (AMC), cefoxitin 30 µg (FOX), ciprofloxacin 5 µg (CIP), nalidixic acid 30 µg (NAL), tetracycline 30 µg (TET), sulfamethoxazole/trimethoprim 23.75/1.25 µg (SXT), streptomycin 10 µg (S), gentamicin 10 µg (CN), imipenem 10 µg (IPM), and chloramphenicol 30 µg (CHL). Inhibition zones were interpreted according to Clinical and Laboratory Standard Institute recommendation (CLSI, 2013). *Escherichia coli* ATCC® 25922 was used for quality control. The *Salmonella* isolates were classified as multidrug-resistance when resistance to at least one agent in three or more antimicrobial classes.

Data analysis

The isolates from the same sample with identical serovars and antimicrobial resistance pattern were considered as one isolate for data calculation and interpretation. Prevalence, serovars and antimicrobials resistance pattern, were described using descriptive statistics. Summary analysis was done at the household level.

RESULTS

Salmonella prevalence and serovars

The household prevalence of *Salmonella* among backyard pigs in Chiang Mai was 21.5% (20/93) (Table 1). The highest prevalence was found in the southern zone (30.3%), followed by central zone (22.2%) and northern zone (12.1%). Among the 15 districts, the highest *Salmonella* detection (75%) was in Doi Lo district. There was no *Salmonella* positive sample recovered in 4 districts; Chiang Dao, Phrao, Saraphi and Omkoi. There were 29 isolates recovered from 20 positive household samples. Nine *Salmonella* serovars were identified among the recovered isolates. The most common serovars from the backyard pigs were *S.* I 4,5,12:i:- (31.0%), followed by *S.* Weltevreden (17.2%), *S.* Rissen (13.8%), *S.* Bovismorbificans (10.3%), and *S.* Stanley (10.3%). The distribution of *Salmonella* serovars by district were shown in Table 1. *S.* Weltevreden was detected in all three zones of Chiang Mai. Whereas, *S.* I 4,5,12:i:- and *S.* III 61:1,v:1,5,(7) was found only in the southern zone. *S.* Albany and *S.* Agona were observed only in the central zone.

Antimicrobial resistance of *Salmonella*

Twenty-one (72.4%) of 29 *Salmonella* isolates were resistant to at least one antimicrobial agent. *Salmonella* isolates were mostly resistant to streptomycin (62.1%) followed by tetracycline (55.2%), ampicillin (48.3%) and sulfamethoxazole/trimethoprim (31.0%). All isolates were susceptible to cefoxitin and imipenem. Antimicrobial resistance by *Salmonella* serovars isolated is shown in Table 2. No antimicrobial resistance was detected among eight isolates from four serovars. This included *S.* I 4,5,12:i:- (1), *S.* Weltevreden (5), *S.* Bovismorbificans (1) and *S.* Stanley (1). Additionally, *S.* Weltevreden was only susceptible to all antimicrobial agents on the panel. Multidrug-resistance was observed among 16 of 29 (55.2%) isolates. Ten different multidrug-resistant patterns were observed as shown in Table 3. The most frequently found multidrug-resistant pattern was AMP-TET-SXT-S. Among multidrug-resistant *Salmonella* serovars, *S.* Agona was resistant to the most antimicrobial agents with the resistance profile AMP-CIP-TET-SXT-S-CHL. *S.* Rissen shared a common multidrug-resistance profile to ampicillin, tetracycline and streptomycin, while *S.* I 4,5,12:i:- shared a common resistance profile to tetracycline and streptomycin than other antimicrobials.

Table 1 Prevalence and serovars of *Salmonella* isolated from backyard pigs in Chiang Mai

Zones	Districts	*Salmonella* Positive (%)	95% CI	Serovars (no. of isolates)
North	Chiang Dao	0/7 (0.0%)	0.0-40.9	-
	Phrao	0/8 (0.0%)	0.0-36.9	-
	Mae Taeng	1/7 (14.2%)	0.4-57.9	*S.* Stanley (1)
	Mae Rim	1/3 (33.3%)	0.8-90.6	*S.* Stanley (1)
	Mai Ai	2/8 (25.0%)	3.2-65.1	*S.* Rissen (1), *S.* Weltevreden (1)
	Total	4/33 (12.1%)	3.4-28.2	
Central	Doi Saket	1/2 (50.0%)	1.3-98.7	*S.* Albany (1)
	San Kanphaeng	2/7 (28.5%)	3.7-71.0	*S.* Agona (1), *S.* Bovismorbificans (3), *S.* Rissen (1)
	San Pa Tong	1/8 (12.5%)	0.3-52.7	*S.* Rissen (1)
	Saraphi	0/2 (0.0%)	0.0-84.2	-
	Hang Dong	2/8 (25.0%)	3.2-65.1	*S.* Rissen (2), *S.* Weltevreden (1)
	Total	6/27 (22.2%)	8.6-42.3	
South	Chom Thong	2/7 (28.5%)	3.7-71.0	*S.* I 4,5,12:i:- (1), *S.* Weltevreden (1)
	Doi Tao	3/8 (37.5%)	8.5-75.5	*S.* I 4,5,12:i:- (6)
	Doi Lo	3/4 (75.0%)	6.7-93.2	*S.* III 61:1,v:1,5,(7) (1), *S.* I 4,5,12:i:- (2), *S.* Heidelberg (1), *S.* Weltevreden (1)
	Omkoi	0/8 (0.0%)	0.3-52.7	-
	Hot	2/6 (33.3%)	4.3-77.7	*S.* Stanley (1), *S.* Weltevreden (1)
	Total	10/33 (30.3%)	15.6-48.7	
Overall		20/93 (21.5%)	13.7-31.2	(29)

Table 2 Antimicrobial resistance profile of *Salmonella* serovars isolated from backyard pigs.

Serovar	*n*	Antimicrobials[1]											No. of antimicrobials[2]		
		TET	AMP	AMC	FOX	CIP	NAL	SXT	S	CN	IPM	CHL	0	1-2	≥3
S. I 4,5,12:i:-	9	8	3	0	0	2	0	5	8	5	0	2	1	0	8
S. Weltevreden	5	0	0	0	0	0	0	0	0	0	0	0	5	0	0
S. Rissen	4	3	4	0	0	0	0	2	4	0	0	0	0	1	3
S. Bovismorbificans	3	1	1	0	0	0	0	1	2	0	0	0	1	1	1
S. Stanley	3	1	2	0	0	0	0	0	0	0	0	0	1	2	0
S. Agona	2	2	2	0	0	1	0	1	1	0	0	2	0	0	2
S. Heidelberg	1	1	1	0	0	0	0	0	1	1	0	0	0	0	1
S. Albany	1	0	1	1	0	0	1	0	1	0	0	0	0	0	1
S. III 61:1,v:1,5,(7)	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0
Total	29	16	14	1	0	3	1	9	18	6	0	4	8	5	16

[1] Numbers under the different antimicrobials indicate the number of resistant isolates.
[2] 0 = Susceptible to all tested antimicrobials; 1–2 = resistant to 1–2 antimicrobials; ≥ 3 = multiresistant to ≥ 3 antimicrobials
TET = Tetracycline, AMP = Ampicillin, AMC = Amoxicillin/clavulanic acid, FOX = Cefoxitin, CIP = Ciprofloxacin, NAL = Nalidixic acid, SXT = Sulfamethoxazole/trimethoprim, S = Streptomycin, CN = Gentamicin, IPM = Imipenem, and CHL = Chloramphenicol

Table 3 Multidrug-resistance patterns of *Salmonella* serovars.

Resistance patterns	*Salmonella* serovars (n)	No. of isolates (n = 16)
AMP-TET-S	*S.* I 4,5,12:i:- (1), *S.* Rissen (1)	2
AMP-TET-CHL	*S.* Agona (1)	1
AMP-TET-SXT-S	*S.* Bovismorbificans (1), *S.* Rissen (2)	3
AMP-AMC-NAL-S	*S.* Albany (1)	1
AMP-TET-S-CHL	*S.* I 4,5,12:i:- (1)	1
AMP-TET-S-CN	*S.* I 4,5,12:i:- (1), *S.* Heidelberg (1)	2
TET-SXT-S-CHL	*S.* I 4,5,12:i:- (1)	1
TET-SXT-S-CN	*S.* I 4,5,12:i:- (2)	2
CIP-TET-SXT-S-CN	*S.* I 4,5,12:i:- (2)	2
AMP-CIP-TET-SXT-S-CHL	*S.* Agona (1)	1

AMP = Ampicillin, AMC = Amoxicillin/clavulanic acid, CIP = Ciprofloxacin, TET = Tetracycline, SXT = Sulfamethoxazole/trimethoprim, S = Streptomycin, CN = Gentamicin, and CHL = Chloramphenicol

DISCUSSION

Twenty-one percent of households with backyard pigs yielded *Salmonella* in this random cross-sectional sample. This is higher than previously reported studies in Thailand which ranged from 2-20% (Chalermchaikit, 2001; Hanson et al., 2002). Compared to other studies in other countries, the prevalence of *Salmonella* in backyard pigs from our study (21.5%) was lower than that reported previously in Hanoi, Vietnam (43.2%) (Thai, 2007) and in Central Vietnam (71.0%) (Lettini et al., 2016) but higher than reported from central Chile (8%) (Alegria et al., 2017b). Various factors could explain the differences of *Salmonella* prevalence in each study such as differences in management, biosecurity practices, times, diagnostic protocols, and locations.

S. I 4,5,12:i:- and *S.* Weltevreden were the most prevalent serovars found in backyard pigs in Chiang Mai. This results are similar to serovars found in rural and industrial pig farms in Central Vietnam (Lettini et al., 2016). Likewise, Tran et al., (2004) has documented *S.* Weltevreden as the most common serovar in both small-scale and commercial pig farms in the Mekong Delta. However, the serovars found in this study were different to the serovars reported previously in backyard pig in Mukdahan province, Thailand which including *S.* Brunei, *S.* Haardt and *S.* Istanbul (Chalermchaikit, 2001). This suggested the dynamic nature of *Salmonella* by time and location. In 2017, *S.* I 4,5,12:i:-, *S.* Weltevreden, *S.* Stanley and *S.* Enteritidis were reported as the top four serovars isolated from patients in Thailand (National Institute of Health of Thailand, 2017). Similarly, other serovars found in this study such as *S.* Rissen, *S.* Bovismorbificans and *S.* Agona have been reported as the 25 most common serovars of *Salmonella* isolated from humans with salmonellosis in Thailand during 1993–2002 (Bangtrakulnonth, 2004). In addition, *S.* Bovismorbificans has progressively been isolated from human patients in Europe (Rimhanen-Finne et al., 2011). The serovars isolated in our study mirror the human serovars isolated in human patients from Thailand documenting that human and backyard pigs likely are sharing similar Salmonella serovars.

Likely inadequate biosecurity practices in backyard or small-scale pig production system perpetuate *Salmonella* survival in the environment shared by humans and animals. Almost half of the backyard pigs in this study were raised on the soil floor. Likely, once *Salmonella* is introduced into the raising area, it is very difficult to eliminate by routine cleaning and disinfection practices. Some farmers never use disinfectants, have no proper sewage removal or treatment, and often raise other animals in the same area. These could be the reasons why the specific *Salmonella* serovars are found in specific areas and serve as on-going sources of infection. These ongoing source serve as potential reservoirs of infection for the pigs, other animals and humans. It was also observed that many of the backyard pigs from this study are fed with leftover food. This could also serve as a possible source of horizontal transmission of *Salmonella* from humans back to the backyard pigs. This highlights the value of good biosecurity practices for backyard pigs and the value of community farmer education about ways to raise pigs safely and minimize disease transmission from pigs to humans and humans to pigs.

Interestingly, this is the first report of *S.* IIIb 61:1,v:1,5,(7) from backyard pigs in Chiang Mai. The serovar is in subspecies diarizonae (IIIb), which generally found in cold-blood animals, sheep, and sometimes causes human illness (Stokar-Regenscheit, 2017). Likely backyard pigs contacted this serovar from wild animals, such as wild birds, rodents, reptiles or insects, which are more likely carriers of this *Salmonella* serovar (Fierer and Guiney, 2001; Manning et al., 2015). Predominant serovars can varies between different geographic locations (Herikstad et al., 2002, Guibourdenche et al., 2010).

A high percentage of *Salmonella* isolates resistant to 62.1% streptomycin, 55.2% tetracycline, 48.3% ampicillin and 31 % sulfamethoxazole. This is comparable to a previous report in Thailand (Love et al., 2015) and other Asian countries (Yang et al., 2010, Lettini et al., 2016). However, isolates from rural pig farms located in Mukdahan, Thailand had a low percentage of antimicrobial resistance (28.6%, 2/7 isolates). The percentage of resistance was 14.3% chloramphenicol, 14.3% kanamycin, 28.6% tetracycline, 28.6% nalidixic acid, 14.3% ciprofloxacin and 28.6% furazolidone (Chalermchaikit, 2001). In other previous studies in Thailand, 14.3-18.0% of backyard pigs carried multidrug-resistant Salmonella (Chalermchaikit, 2001; Love et al., 2015). In our study, *S.* Weltevreden was the only serovar that all isolates were susceptible to all antimicrobials on the panel. This is a similar finding as reported by Aarestrup et al., (2003.) which noted a low frequency (48/503; 9.5%) of resistance among *S.* Weltevreden isolated from humans and other reservoirs in South-East Asia. The authors suggested that the serovar likely does not easily acquire resistance or that the natural reservoirs were not exposed to antimicrobial agents.

Previous studies reported that the sources of multidrug-resistant *Salmonella* present in backyard pigs can be affected by the herd size and antimicrobial usage on the farm. The most common antimicrobial resistance pattern profile in this study was AMP-TET-SXT-S. This was similarly reported by Phongaran et al. (2019.) They documented that the most frequent pattern isolated from pig feces collected from slaughterhouses in nine provinces of Thailand was AMP-SXT-TET. In our study, it was noted that among the farms that recently use antimicrobials, some used antimicrobials without a prescription from veterinarians and some producers were unaware of the antimicrobial withdrawal time. Almost half of participates in backyard pig production used commercial feed. As suggested by Love et al.(2015) commercial medicated feed is likely related to the development of antimicrobial resistance. Often the farmer was not aware of the type and dose of antimicrobials that was mixed in the feed. Again, highlighting the value of backyard pig owner education.

CONCLUSION

The three most prevalent serovars circulating in backyard pigs in Chiang Mai, Thailand were *S.* I 4,5,12:i:-, *S.* Weltevreden, and, *S.* Rissen.The different serovars were isolated with a broad range of antimicrobial resistance profiles. The backyard pigs could serve as a reservoir of *Salmonella* that could infect and transmit antimicrobial resistance genes to humans or other animals. Good biosecurity practices and educating backyard pig owners are importance to raise pigs safely and minimize the risk of *Salmonella* transmission in the communities.

CONFLICT of INTEREST

All authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: M.I.
Sample collection: C.A. S.N.
Performed the laboratory analysis: C.A. N.A. M.I.
Analyzed the data: N.A. M.I.
Contributed reagents/materials/analysis tools: N.A. M.I.
Wrote the paper (review and editing): C.A. N.A. M.I. J.B.

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