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Research article

Prevalence, genetic characterization, and antimicrobial resistance of *Salmonella* isolated from meat goats in the Northeastern region of Thailand

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Abstract

This study aimed to determine the prevalence, genotypic diversity, and antimicrobial resistance pattern of *Salmonella* isolated from meat goats in the Northeastern region of Thailand. A total of 1,014 rectal swabs were collected from 30 meat goat farms during April to November, 2018. *Salmonella* was isolated and identified according to the International Organization for Standardization protocol (ISO-6579:2002/AMD:2017) and serotyped using a slide agglutination test following the Kauffmann-White scheme. An antimicrobial susceptibility test to determine minimal inhibitory concentration (MIC) of 12 antimicrobial agents was performed using a broth microdilution method following the CLSI protocol (2017). Pulsed-field gel electrophoresis (PFGE) of *XbaI* digested chromosomal DNA was used to determine genotypic diversity of the isolates. The overall prevalence of *Salmonella* in the meat goats was 1.28%. A total of 13 *Salmonella* isolates recovered from the meat goats belonged to 4 serovars including *S.* Weltevreden (n=4), *S.* Bovismorbificans (n=4), *S.* Paratyphi B (n=4), and *S.* Stanley (n=1). Antimicrobial susceptibility testing revealed 2 antibiogram patterns. Eleven *Salmonella* isolates were susceptible to all antimicrobial agents tested, except sulfamethoxazole, and the other 2 isolates were susceptible to all antimicrobials. Genetic characterization of 13 *Salmonella* isolates by PFGE revealed 9 PFGE patterns that were grouped into 4 major clusters, A, B, C and D, with an 80% similarity value. This study revealed a low prevalence of *Salmonella* in meat goats in the Northeastern region of Thailand. *Salmonella* isolates were susceptible to most antimicrobials tested, with a very high proportion of resistance to sulfamethoxazole being observed.

Keywords: Antimicrobial resistance, Meat goats, Prevalence, *Salmonella*, Thailand

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INTRODUCTION

Salmonella are pathogenic bacteria that live in the gastrointestinal tract, causing the most common zoonotic bacterial disease in humans that presents as gastroenteritis (Abulreesh, 2012; Hoelzer et al., 2011; Rodriguez et al., 2006). *Salmonella* infections (salmonellosis) are considered the most common food-borne disease and have been recognized globally in both developed and developing countries because of high morbidity and economic burden (Igbinosa, 2015; Lampang et al., 2014; Saha et al., 2013). In the U.S., *Salmonella* is the leading foodborne pathogen, causing the largest number of deaths and the highest medical cost burden (Batz et al., 2012). The annual cost associated with salmonellosis for 2010 was estimated at $2.71 billion from 1.4 million cases (USDA, 2013). *Salmonella* infections in humans mostly occurred through the consumption of contaminated animal products such as eggs, meats and milk, or green vegetables contaminated by manures (Sodagari et al., 2020). Human cases also occur when individuals have direct contact with the infected animals, including pets (Anuchatkitcharoen et al., 2020; Hoelzer et al., 2011). *Salmonella* infected animals shed the organism in their feces from where it can spread into soil, water, crops and/or other animals (Mathole et al., 2017; WHO, 2013; Nouichi et al., 2009; Patchanee et al., 2016). The disease can affect all species of domestic animals, with young, pregnant, and lactating animals being most susceptible (Demirbilek, 2018). Enteric disease is the most common clinical manifestation (Demirbilek, 2018). However, a wide range of clinical signs which include acute septicemia, abortion, arthritis, and respiratory disease may be seen (Demirbilek, 2018). Many animals, especially pigs and poultry, may be infected but show no clinical illness (Demirbilek, 2018). Such animals may be important in the spread of infection between flocks and herds and as sources of food contamination and human infection (Igbinosa, 2015; OIE, 2018).

Antimicrobial resistance of *Salmonella* is a global public and animal health concern (Elkenany et al., 2019; Nouichi et al., 2009; Umeh and Enwuru, 2014; WHO, 2013). Antimicrobials are used in food-producing animals for prophylaxis and treatment of bacterial infections, as well as for enhancing the growth of farm animals. The widespread administration of medically important antimicrobials to food-producing animals at subtherapeutic or prophylactic doses may promote on-farm selection of antimicrobial resistant strains. Thus, the risks to human health from consumption of meat products containing antimicrobial resistant *Salmonella* can be increased. (Economou and Gousia, 2015; Ferede et al., 2015; Mwanyika et al., 2016; Wang et al., 2019; Zelalem et al., 2011). The digestive tract of animals persists as the major reservoir of *Salmonella* and plays a role in the distribution of salmonellosis (Nisbet and Ziprin, 2001). The evisceration process during meat production is the leading source of carcass contamination with *Salmonella*; fecal bacteria may accidentally contaminate the meat and meat products (Andino and Hanning, 2015; Vidayanti et al., 2021; Zare et al., 2014). Animals have been implicated as a source of human infection with antimicrobial resistant *Salmonella*. (Ferede et al., 2015; Zelalem et al., 2011; Zewdu and Cornelius, 2009). Antimicrobial resistant *Salmonella* in food animals that acquire antimicrobial resistance genes during husbandry might lead to human infections through the food chain (Glenn et al., 2011). In addition, they could perpetuate the spread of antimicrobial resistance genes to humans by horizontal gene transfer through mobile genetic elements such as plasmids and integrons (Igbinosa, 2015). Class 1 integrons, carrying

resistance genes against many antimicrobial classes in various pathogens, are considered as one of most common contributors to multidrug resistant bacterial infections globally (Stokes et al., 2006).

Since the early 1990s, *Salmonella* strains resistant to a range of antimicrobials have emerged (Umeh and Enwuru, 2014). Increase of antimicrobial resistance, prevalence, virulence, and adaptability of *Salmonella* are now a serious public health concern worldwide (Bantawa et al., 2019; Hong et al., 2016; Ketkhao et al.; 2019). The burden of food-borne disease is increasing due to antimicrobial resistance, which poses a greater risk of treatment failure (Vidayanti et al., 2021). The number of people facing antimicrobial resistance in the U.S. is still high: more than 2.8 million antibiotic-resistant infections occur in the U.S. each year, and more than 35,000 people die as a result (CDC, 2019). Several studies have shown the presence of *Salmonella* in food-producing animals and animal products in many parts of the world (Padungtod and Kaneene, 2006; Jajere et al., 2016). Meat goats have also been implicated as a source of *Salmonella* food poisoning (Duffy et al., 2009; Chandra et al., 2006). However, there is little information on the carriage of *Salmonella* in meat goats. In Thailand, the epidemiological investigation of *Salmonella* infections in meat goats has not been studied and there has been no report on the status of prevalence, genotypic diversity, or antimicrobial susceptibility of *Salmonella* in meat goats. Thus, the objectives of this study were to estimate the prevalence, genotypic diversity, and antimicrobial resistance profiles of *Salmonella* isolates from apparently healthy goats in the Northeastern region of Thailand.

MATERIALS and METHODS

Sample collection

A cross-sectional study was conducted at 30 meat goat farms located in 7 provinces in the northeastern region of Thailand (Figure 1): Khon Kaen (n=3), Udon Thani (n=3), Loei (n=3), Nong Khai (n=5), Nakhon Ratchasima (n=4), Chaiyaphum (n=5), and Burirum (n=7) from May to November, 2018. The study areas were chosen based on the location and meat goat population. Four provinces including Khon Kaen, Unidonthai, Loei and Nong Khai are located in the northern part of the northeastern region, and another 3 provinces, Nakhon Ratchasima, Chaiyaphum and Burirum, are located in the southern part of this region. Meat goat populations in these provinces, except Loei, are larger than other provinces within the same region (DLD, 2017). The sample size of this study was determined by using the formula $n = Z^2PQ/L^2$ (Lwanga and Lemeshow, 1991), with an expected prevalence of 2% based on the result of a pilot study, and 1% of allowable error. The calculated sample size was 753 samples. However, 1,014 samples were finally collected to obtain a sufficient number of *Salmonella* isolates for investigation of genotypic diversity. A convenient sampling method was used for this study. In each province, at least 3 farms that had more than 10 goats were selected; all apparently healthy goats of at least 3 months of age, both male and female, in the farms were included in the study. There was difficulty in collecting fresh feces from meat goats reared in open-range conditions. Thus, the rectal swab method was chosen for sample collection, which requires the least amount of restraint of animals.

Rectal swab samples were aseptically collected using sterile cotton swab and put into sterile universal bottles containing Cary-Blair transport medium (Oxoid, England). Samples were kept at 4ºC in an ice box during transportation to the laboratory. This study was approved by the Animal Ethics Committee of the Institutional Animal Care and Use of Khon Kaen University based on the Ethics of Animal Experimentation National Research Council of Thailand. (IACUC-KKU-18/61).

Isolation and identification of Salmonella

Salmonella was isolated from rectal swab samples using the protocol adapted from the International Organization for Standardization (ISO-6579, 2002/AMD1:2017). Briefly, the swab samples were pre-enriched in buffered peptone water (BPW, Difco, France) and incubated at 37°C for 18±2 h. One hundred microliters of the pre-enriched sample were transferred into Modified Semi-solid Rappaport-Vassiliadis agar (MSRV, Difco, France) and incubated at 42°C for 24±3 h. A loop full of inoculum from each MSRV agar culture was streaked onto Xylose Lysine Deoxycholate (XLD, Difco, France) and Brilliant Green Agar plates (BGA, Difco, France) and incubated at 37°C for 24±3 h. Five typical or suspected colonies of *Salmonella* were then selected from the culture plates, further streaked onto the surface of pre-dried nutrient agar plates (Difco, France), and then incubated at 37°C for 24 ±3 h. *Salmonella* spp. were identified by biochemical tests using triple sugar iron agar, L-lysine decarboxylation medium, urease, and indole production tests (Difco, France).

Serotyping

Serotyping of the *Salmonella* isolates based on the somatic (O) and flagella (H) antigens was performed using a slide agglutination test following the Kauffmann-White scheme (Popoff and Le Minor, 2007; Grimont and Weill, 2007). Each isolate was tested for autoagglutination with 0.85% normal saline solution prior to serotyping. The *Salmonella* isolates were then tested against antisera to the O and H antigens. For O-typing, the isolates were tested with *Salmonella* polyvalent "O" antiserum (OMA, OMB, OMC, OMD, OME, OMF and OMG) and *Salmonella* antiserum group (group A, B, C, D, E and I), respectively. For H-typing, polyvalent "H" antiserum (HMA, HMB, HMC, HMD and HMIII) was used for testing. For H- positive isolates, phase inversion was done prior to detection of 2 H-antigen phase. The results of both O and H -typing were combined to determine the *Salmonella* serovar using the Kauffmann-White scheme.

Antimicrobial susceptibility testing (AST)

The antimicrobial susceptibility testing was performed on all *Salmonella* isolates to determine the minimal inhibitory concentration (MIC) of 12 antimicrobial agents (Sigma, USA), including ampicillin, ceftazidime, cefotaxime, meropenem, gentamicin, chloramphenicol, tetracycline, ciprofloxacin, colistin, sulfamethoxazole, trimethoprim and nalidixic acid using a broth microdilution method following the Clinical & Laboratory Standards Institute (CLSI) protocol (CLSI, 2017a; CLSI, 2017b). *Escherichia coli* ATCC25922 and *Enterococcus faecalis* ATCC 29212 were used as quality control organisms. A 10^6 CFU/ml bacterial suspension was inoculated into a 96 well microtiter plate containing 2-fold dilutions of antimicrobial agents. Plate

counts were conducted to confirm the concentration of each inoculum in the susceptibility testing. After incubation at 37°C for 18-24 h, the minimal inhibitory concentration (MIC) of each antimicrobial agent was determined by observing bacterial growth in each well, and the lowest concentration that completely inhibited bacterial growth was determined as the MIC. The MIC breakpoints provided by CLSI were used to categorize *Salmonella* isolates as resistant or susceptible (CLSI, 2017a; CLSI, 2017b).

Pulsed-field gel electrophoresis (PFGE)

Pulsed-field gel electrophoresis (PFGE) was performed according to the standard operating procedure for PulseNet PFGE of *E. coli* O157:H7, *E. coli* non-O157 (STEC), *Salmonella* serotypes, *Shigella sonnei*, and *Shigella flexneri* by the CDC (2017) and the previously described method by Nsofor (2016). Restriction endonuclease digestion was carried out using *Xba*I (Invitrogen, USA) at 37°C for 2 h. *Salmonella* Braenderup H9812 was included as the molecular weight size standard. Fragments were separated in 1% agarose gel using CHEF-DR III apparatus (Bio-Rad, USA) with pulse times of 2.2-63.8s at 14°C for 18 h, and at 6 V/cm in TBE buffer. After electrophoresis, the agarose gels were stained with 0.1 µg/ml ethidium bromide and DNA fragments were visualized under ultraviolet light in a gel documentation system (GelDoc-It, USA).

Data collection and statistical analysis

Statistical analysis was carried out using Statistical Package for the Social Science (SPSS) software version 17.0 (IBM, USA). Descriptive statistics were used to determine the prevalence of *Salmonella* in the study area and antimicrobial resistance patterns. Differences in prevalence of *Salmonella* among provinces were analyzed using Fisher's exact test. A statistically significant difference among variables was considered to exist if the calculated *p*-value was less than 0.05. The PFGE profiles were analyzed using BioProfile software (Vilber Lourmat, Germany) with the dice coefficient similarity index, and a dendrogram was then constructed using the unweighted pair group match average (UPGMA) method.

RESULTS

Salmonella prevalence and serovars

Out of 1,014 rectal swab samples examined, 13 *Salmonella* isolates were recovered. These isolates belonged to 4 serovars: *S.* Weltevreden (n=4), *S.* Bovismorbificans (n=4), *S.* Paratyphi B (n=4) and *S.* Stanley (n=1) as shown in Table 1. *Salmonella* isolates were obtained from meat goats raised in 9 farms located in 6 provinces (Fig. 1). Four provinces including Khon Kaen, Udon Thani, Loei, Nakhon Ratchasima had one positive farm. Nong Khai and Chaiyaphum had 2 and 3 positive farms, respectively. In Buriram province, no sample was positive for *Salmonella*. The overall prevalence of *Salmonella* in meat goats of these study areas was 1.28% (13/1014), and prevalence in each province and each farm were in the ranges of 0-3.28% and 0-14.29%, respectively (Table 1 and Figure 1). The prevalence of *Salmonella* among the provinces were not significantly different ($P > 0.05$).

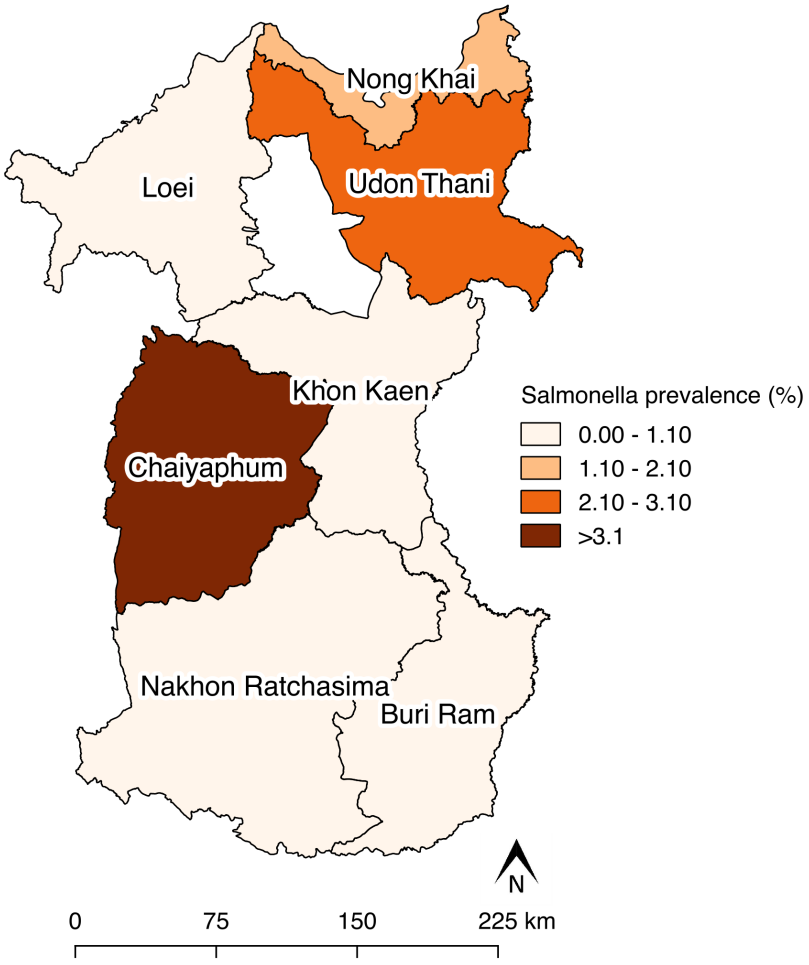

Figure 1 Geographic location of 7 provinces where the farms in this study are located. The prevalence of *Salmonella* is represented by different color.

Table 1 Results of isolation, serotyping, and prevalence of *Salmonella* in the meat goat farms in the Northeastern region of Thailand.

Province	Farm No.	Number of Rectal swab samples	Number of positive samples	Prevalence (%)	95%CI	Serovars (Isolate number)
Khon Kaen	1	67	1	1.49	0.26-7.98	*S.* Weltevreden (no.1)
	2	10	0	0		
	3	44	0	0		
	total	121	1	0.83	0.14-4.53	
Udon Thani	1	75	3	4.00	1.36-11.11	*S.* Bovismorbificans (no.4, no.5)
	2	13	0	0		*S.* Stanley (no.6)
	3	10	0	0		
	total	98	3	3.06	1.04-8.61	
Loei	1	132	1	0.76	0.13-4.16	*S.* Weltevreden (no.9)
	2	47	0	0		
	3	25	0	0		
	total	204	1	0.49	0.08-2.72	
Nong Khai	1	32	0	0		
	2	24	0	0		
	3	21	0	0		
	4	17	1	5.88	1.04-26.98	*S.* Paratyphi B (no.7)
	5	25	1	4.00	0.70-19.54	*S.* Weltevreden (no.8)
	total	119	2	1.68	0.46-5.92	
Nakhon Ratchasima	1	56	2	3.57	0.98-12.11	*S.* Bovismorbificans (no.2, no.3)
	2	35	0	0		
	3	57	0	0		
	4	50	0	0		
	total	198	2	1.01	0.27-3.60	
Chaiyaphum	1	50	0	0		
	2	26	1	3.85	0.68-18.89	*S.* Weltevreden (no.12)
	3	22	1	4.55	0.80-21.79	*S.* Paratyphi B (no.13)
	4	14	2	14.29	4.00-39.94	*S.* Paratyphi B (no.10, no.11)
	5	10	0	0		
	total	122	4	3.28	1.28-8.12	
Burirum	1	28	0	0		
	2	25	0	0		
	3	18	0	0		
	4	15	0	0		
	5	17	0	0		
	6	26	0	0		
	7	23	0	0		
	total	152	0	0		
Grand total	30	1,014	13	1.28	0.75-2.18	

Antimicrobial susceptibility patterns

Antimicrobial susceptibility testing revealed 2 antibiogram patterns: susceptible to all antimicrobials tested, and resistant to only sulfamethoxazole (Table 2). Two out of 13 (15.38%) *Salmonella* isolates identified as *S.* Weltevreden were not resistant to any antimicrobial agents. Eleven *Salmonella* isolates (84.62%, 11/13) which belonged to *S.* Weltevreden, *S.* Bovismorbificans, *S.* Paratyphi B and *S.* Stanley, were susceptible to ampicillin (MIC_{50}=1 mg/l, MIC_{90}=1 mg/l), chloramphenicol (MIC_{50}=4 mg/l, MIC_{90}=8 mg/l), ceftazidime (MIC_{50}=0.25 mg/l, MIC_{90}=0.5 mg/l), gentamicin (MIC_{50}=0.25 mg/l, MIC_{90}=0.5 mg/l), cefotaxime (MIC_{50}=0.03125 mg/l, MIC_{90}=0.0625 mg/l), meropenem (MIC_{50}=0.0156 mg/l, MIC_{90}=0.0156 mg/l), tetracycline (MIC_{50}=2 mg/l, MIC_{90}=2 mg/l), ciprofloxacin (MIC_{50}=0.0156 mg/l, MIC_{90}=0.0312 mg/l), colistin (MIC_{50}=0.5 mg/l, MIC_{90}=1 mg/l), trimethoprim (MIC_{50}=0.5 mg/l, MIC_{90}=0.5 mg/l) and nalidixic acid (MIC_{50}=4 mg/l, MIC_{90}=8 mg/l), but resistant to sulfamethoxazole (MIC_{50}=1024 mg/l, MIC_{90}=1024 mg/l) as shown in Table 2. Multidrug-resistant *Salmonella* were not found.

PFGE profiles

All 13 *Salmonella* isolates were typable by the PFGE technique. PFGE analysis identified 9 distinguishable *Salmonella* genotypes (9 patterns) that grouped into 4 major clusters, A, B, C and D, with an 80% similarity value. A dendrogram based on the 80% similarity value is presented in Figure 2. Cluster A consisted of 3 PFGE patterns (P1-P3) of 4 *S.* Weltevreden isolates from the farms located in 4 different provinces: Khon Kaen, Nong Khai, Loei, and Chaiyaphum. Cluster B was composed of 2 PFGE patterns (P4 and P5) of 4 *S.* Bovismorbificans isolates from a farm in Nakhon Ratchasima (n=2) and a farm in Udon Thani (n=2). Identical PFGE patterns were observed among isolates from the same farm. Three PFGE patterns (P6-P8) of 4 *S.* Paratyphi B isolates from Nong Khai and Chaiyaphum were grouped into cluster C. Cluster D consisted of 1 PFGE pattern (P9) of 1 *S.* Stanley isolate from Udon Thani. *Salmonella* belonging to the same serovar were genetically highly related and showed more than 80% similarity. Isolates obtained from the same farm were more closely related to each other than to the isolates from different farms. Only the P2 PFGE pattern of *S.* Weltevreden was susceptible to sulfamethoxazole.

Table 2 Antimicrobial susceptibility pattern in *Salmonella* isolates from the meat goat farms in the Northeastern region of Thailand.

Isolate number	Serovar	MIC value (mg/l) / Susceptibility result (S/I/R)											
		AMP	CTX	CAZ	MEM	CHL	NAL	CIP	GEN	SUL	TET	TRI	CST
1	*S* .Weltevreden	1/S	0.03125/S	0.5/S	0.0156/S	4/S	4/S	0.008/S	0.125/S	1024/R	2/S	0.5/S	0.5/S
8	*S* . Weltevreden	1/S	0.0625/S	0.25/S	0.0156/S	4/S	4/S	0.0156/S	1/S	1024/R	2/S	0.5/S	1/S
9	*S* . Weltevreden	1/S	0.03125/S	0.25/S	0.0156/S	8/S	8/S	0.0156/S	0.25/S	64/S	2/S	0.5/S	1/S
12	*S* . Weltevreden	1/S	0.03125/S	0.25/S	0.0156/S	8/S	4/S	0.008/S	0.25/S	128/S	2/S	0.25/S	0.5/S
2	*S* . Bovismorbificans	1/S	0.0625/S	0.25/S	0.0156/S	4/S	4/S	0.0312/S	0.5/S	1024/R	2/S	0.5/S	0.5/S
3	*S* . Bovismorbificans	1/S	0.0625/S	0.5/S	0.0156/S	4/S	4/S	0.0312/S	1/S	1024/R	2/S	0.5/S	1/S
4	*S* . Bovismorbificans	1/S	0.0625/S	0.5/S	0.0156/S	4/S	4/S	0.0312/S	0.5/S	1024/R	2/S	0.5/S	1/S
5	*S* . Bovismorbificans	1/S	0.0625/S	0.5/S	0.0156/S	8/S	4/S	0.0312/S	0.25/S	1024/R	2/S	0.5/S	0.5/S
7	*S* . ParatyphiB	1/S	0.0625/S	0.5/S	0.0156/S	8/S	8/S	0.008/S	0.25/S	1024/R	2/S	1/S	1/S
10	*S* . ParatyphiB	1/S	0.03125/S	0.5/S	0.0156/S	4/S	4/S	0.0156/S	0.25/S	512/R	2/S	0.5/S	0.5/S
11	*S* . ParatyphiB	2/S	0.03125/S	0.25/S	0.0156/S	8/S	4/S	0.0156/S	0.25/S	1024/R	2/S	0.5/S	0.5/S
13	*S* . ParatyphiB	1/S	0.03125/S	0.25/S	0.0156/S	8/S	8/S	0.0156/S	0.25/S	1024/R	2/S	0.5/S	0.5/S
6	*S* . Stanley	1/S	0.125/S	0.5/S	0.0156/S	8/S	8/S	0.0312/S	0.5/S	1024/R	4/S	0.25/S	0.5/S

S = susceptible, I = intermediate, R = resistant
AMP = ampicillin, CHL = chloramphenicol, SUL = sulfamethoxazole, CAZ = ceftazidime, NAL = nalidixic acid, TET = tetracycline, CTX = cefotaxime, CIP = ciprofloxacin, TRI = trimethoprim, MEM = meropenem, GEN = gentamicin, CST = colistin

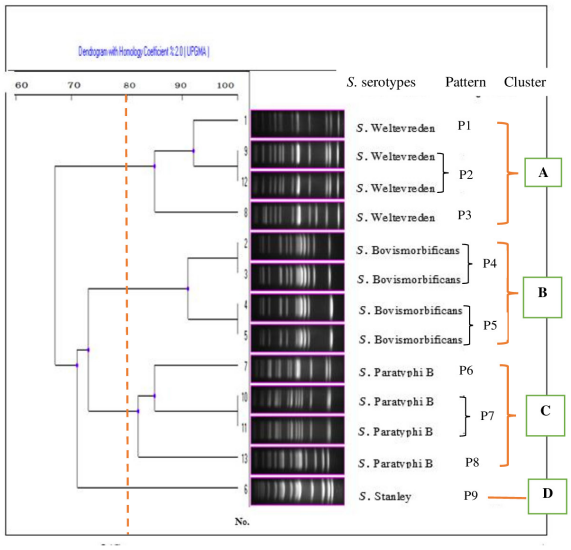

Figure 2 Dendrograms generated using UPGMA algorithms based on PFGE profiles of 13 *Salmonella* isolates recovered from meat goats.

DISCUSSION

This is the first study reporting the prevalence of *Salmonella* from meat goats in Thailand. Our results showed that the overall prevalence of *Salmonella* in meat goats of the study areas was relatively low, at 1.28%. The prevalence of *Salmonella* was found to vary between farms (0-14.29%) and between provinces (0-3.28%), although the prevalence of *Salmonella* among the provinces was not significantly different ($P > 0.05$). The finding of the low prevalence of *Salmonella* in meat goats in our study is in agreement with other studies in several countries worldwide. Davies et al. (2004) reported a very low prevalence of *Salmonella* in sheep and goats in Britain at 0.1%, and the prevalence of *Salmonella* in goats in South Africa was also low at 0.43% (Nouichi and Hamdi, 2009). In Ethiopia, the prevalence of *Salmonella* in slaughtered goats was found to range from 1-3% (Kassaye et al., 2015; Molla et al., 2006). Low prevalence of *Salmonella* was also demonstrated in previous studies conducted in Asian countries. Mahmood et al. (2014) found a low prevalence of *Salmonella* in diarrheic adult goats in the southern district of Lahore, Pakistan, at 0.27%, while Esmaeili and Rahmani (2016) reported the prevalence of *Salmonella* in goat flocks located in Bushehr provinces, Iran, at 1.05%.

In contrast, higher prevalence of *Salmonella* in goats, ranging from 11-46% was shown in other studies. Teklu and Negussie (2011) reported a prevalence of 11.7% for *Salmonella* in goats at an export abattoir in Ethiopia, and Ferede et al. (2015) reported a prevalence of 17.7% in apparently healthy

goats at Dire Dawa municipal abattoir, Eastern Ethiopia. Similar prevalence of *Salmonella* in goats and goat meat was reported in Nigeria, where prevalence of *Salmonella* infections in goats was found at 17.6% (Chandra et al., 2006); 10% of goat meat sampled from retail shops in Maiduguri were shown to contain *Salmonella* (Musa et al., 2017). In the Asian country of Bangladesh, the prevalence of *Salmonella* associated with goats was reported at 12.76% (Saha et al., 2013). Interestingly, a moderately high prevalence of *Salmonella* in goats was found in previous studies in Australia. A prevalence of 26.5% was reported in Western Australian rangeland goats (Al-Habsi et al., 2018), and Duffy et al. (2009) reported the prevalence of *Salmonella* in carcass swabs, rumen, and feces from goats at two Australian abattoirs to be at 28.9% (35/121), 45.5% (55/121) and 46.3% (56/121), respectively. Differences or similarity in farming practice, healthy or diseased state, type of sample, and/or isolation method may be responsible for prevalence observed in the reported studies. The possible reasons for the low prevalence reported in our study might be because the samples were collected from apparently healthy goats and the studied farms had good sanitation and hygiene practices. *Salmonella* infections are related to management issues and their control depends on controlling the source of contamination and transmission (Mathole et al., 2017). In addition, the use of rectal swab samples containing small amounts of feces in this study may also lead to low prevalence result.

The most common *Salmonella* serotypes identified in this study were *S.* Weltevreden, *S.* Bovismorbificans and *S.* Paratyphi B, which was similar to the report in India by Chandra et al. (2006). However, different serotypes including *S.* Typhimurium, *S.* Poona, *S.* Chester, *S.* Abortus-ovis and *S.* Saintpaul, were recovered from goats in other studies (Esmaeili and Rahmani, 2016, Mathole et al., 2017). *S.* Paratyphi B and *S.* Weltevreden are the important serotypes from a public health point of view. Both serovars have been classified in the emerging group of *Salmonella* serovars worldwide and are potential risks to meat handlers as well as consumers due to their zoonotic nature (Chandra et al., 2006). *S.* Paratyphi B is a causative agent of paratyphoid fever, and is endemic in Thailand with considerable regional variation, making it a public health concern. The trend in the occurrence of paratyphoid fever remains stable, with the peak incidence of 0.77 cases per 100,000 persons being observed in 2009 (Techasaensiri et al., 2018). Our study indicated that *S.* Paratyphi B and *S.* Weltevreden may be circulating in meat goat farms, posing a health risk to people who have close contact with the goats.

Antimicrobial susceptibility testing (AST) of the *Salmonella* isolates obtained in this study revealed that all isolates were still susceptible to almost all antimicrobials, except sulfamethoxazole. No isolate was identified as multidrug resistant (MDR). High resistance to sulfamethoxazole observed in our study was similar to the study by Saha et al. (2013) who reported that *Salmonella* strains associated with goats in Bangladesh were resistance to sulfamethoxazole at a rate of 66.7%. Most *Salmonella* isolated from goats in the studies in Tanzania and Australia were also found to be susceptible to most antimicrobials tested (Mwanyika et al., 2016; Al-Habsi et al., 2018). In contrast, reports of other researchers from different areas showed that *Salmonella* isolates were resistant to commonly used antimicrobials including tetracycline, nitrofurans, streptomycin,

kanamycin, and ampicillin, and were resistance to multiple antimicrobials (Saha et al., 2013; Zelalem et al., 2011; Zewdu and Cornelius, 2009; Glenn et al., 2011; Bantawa et al., 2019). High rates of resistance to ampicillin (54.5%), amoxicillin (45.5%), streptomycin (81.8%), sulfonamide (42%) and Trimethoprim (75%) were also reported in *Salmonella* recovered from goat meats in eastern Ethiopia (Ferede et al., 2015; Ferede et al., 2017). The differences in antimicrobial resistance among different studies could be due to the use of antimicrobial agents in animals at subtherapeutic levels or prophylactic doses, or to indiscriminate use of antimicrobials (Mwanyika et al., 2016; Glenn et al., 2011; Mahmood et al., 2014). Differences among serovars with respect to antimicrobial resistance have been documented. According to a NARMS report in 2010, the serovars with greater resistance to antimicrobials are Typhimurium with specific resistance to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole/sulfisoxazole, and tetracycline (ACSSuT), and Enteritidis with resistance to nalidixic acid (John et al., 2011). Serovars Newport, Heidelberg, and Dublin were also shown to be resistant to various antimicrobial groups. In terms of multidrug resistance, the most prevalent serovars of epidemiological importance are Typhimurium, Heidelberg, Dublin and Paratyphi B. In this study, the 4 serovars including *S.* Weltevreden, *S.* Bovismorbificans, *S.* Paratyphi B and *S.* Stanley from 11 samples were resistant to only sulfamethoxazole. This result could be because sulfamethoxazole was widely used as an antidiarrheal in breeder goats in the past in Thailand. Although commercially available drugs contain both sulfonamides and trimethoprim, resistance to trimethoprim was not found. Sulfonamides and trimethoprim resistance is mediated via different unshared mechanisms (Sköld, 2001). Therefore, bacteria can develop resistance to each of these drugs individually. It highlights that, in addition to the sulfonamides and trimethoprim combination, the susceptibility to sulfonamides and trimethoprim should be individually determined in antimicrobial resistance monitoring. The possible reasons for high susceptibility to almost all antimicrobials of *Salmonella* isolates in this study may be because the farmers limit the use of antimicrobials in animals; antimicrobials are administered to treat individual cases only. Moreover, antimicrobials have not been used as growth substances in feed in the study farms because goats are quite robust and tolerant to environmental conditions in Thailand.

PFGE and AST are the two commonly used methods for studying microbial epidemiology and trends in the antimicrobial resistance of bacteria. PFGE is currently used by the CDC PulseNet surveillance program and is generally accepted as the "gold standard" for molecular typing of *Salmonella* (Chotinan and Tadee, 2015; Harbottle et al., 2006). Genetic diversity and relatedness among the *Salmonella* isolates in our study were investigated using PFGE. Based on an 80% similarity value, we identified 9 PFGE patterns grouped into 4 major clusters. *Salmonella* isolates within the same serovar were genetically related at more than 80% similarity. Only one PFGE pattern (P2) of *S.* Weltevreden was susceptible to sulfamethoxazole. Identical or closely related PFGE patterns were observed among isolates from the same farm indicating the circulation of some *Salmonella* strains within the farm. The results demonstrated that PFGE delivered good discriminatory power for *Salmonella* identification and could provide valuable information for disease surveillance.

CONCLUSION

This study revealed the low prevalence of *Salmonella* in meat goats in the Northeastern region of Thailand. Several serovars including *S.* Weltevreden, *S.* Bovismorbificans, *S.* Paratyphi B and *S.* Stanley were found in meat goats. Genetic similarity of more than 80% was observed in *Salmonella* isolates belonging to the same serovar. *Salmonella* isolates were susceptible to almost all antimicrobials and highly resistant to only one antimicrobial, sulfamethoxazole. Our results suggested that the risk to consumers from goat meat is low, especially when meat products are cooked prior to consumption. However, the presence of carrier animals in flocks could be a source of environmental contamination and spread. *S.* Paratyphi B was detected in the current study in field conditions, thus the risk of salmonellosis to those who have close contact to animals must be considered. Further studies will need to be conducted to identify the potential source of *Salmonella* infection in meat goats, and epidemiological relationship of *Salmonella* between meat goat farms and retails in Thailand.

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AUTHORS' CONTRIBUTIONS

SA and WC designed the experiment. SA, WC and SK collected the samples, WC performed the experiments, analyzed data and wrote the manuscript. PP analyzed data and revised the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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