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Prevalence and antimicrobial resistance of *Salmonella* spp. isolated from fattening beef cattle at the slaughterhouse in Sakon Nakhon Province

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Abstract

The objective of this study was to determine the prevalence and antimicrobial resistance of *Salmonella* spp. isolated from fattening beef cattle at the slaughterhouse of Phon Yang Kham Livestock Breeding Cooperatives under the National Peacemaking Council in Sakon Nakhon Province. One hundred and twenty samples of bovine cecal contents were collected during November, 2014 – July, 2015 and isolated for *Salmonella* using ISO6579:2002/AMD 1:2007 standard protocol. It was found that the prevalence of *Salmonella* was 0.83% (1 out of 120 samples). Then, the isolated *Salmonella* was submitted to reference laboratory for serovar identification. The result suggested that it was *Salmonella* Brunei, serogroup C. Additionally, the antimicrobial susceptibility of the collected *Salmonella* to 11 antibiotics including ampicillin, cefotaxime, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, penicillin, streptomycin, tetracycline and sulfamethoxazole/trimethoprim was examined by using disk diffusion test. The result showed that this *Salmonella* resisted to ampicillin and penicillin. Although *Salmonella* may not pose a major problem in current fattening beef cattle production, high precaution is needed when ampicillin and penicillin are to be prescribed in case of *Salmonella* infection in fattening beef cattle production.

Keywords: *Salmonella* spp.; Fattening beef cattle; Slaughterhouse; Sakon Nakhon Province

1. Introduction

Salmonella is an infective agent causing important diseases in human digestive tract. It might be found in food, improper food production and unhygienic management of producers. In Thailand, 105,028 people were suffered from food poisoning according to the report on the investigation of food poisoning conducted by Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health in 2012 during 1 January – 26 November 2012 [1]. *Salmonella* is able to transmit and spread to human mainly through contaminated media from environment particularly food which could be contaminated during the production, storage and preservation with

poor sanitary practices. Various dietary products likely contaminated with *Salmonella* are meat, raw milk, butter, chocolate, bean sprouts, cereals and water. Meat and meat products are the important one borne with this disease agent because *Salmonella* is a type of bacteria able to grow inside digestive tract of almost all poultry and livestock such as chicken and pig. According to the study on *Salmonella* contamination in meat derived from slaughterhouses throughout Thailand in 2006 – 2008 reported by Bureau of Health and Hygiene 1 – 9, it was found that in 2006, 1884 meat samples out of 4014 (46.93%) were contaminated with *Salmonella* and in 2007, *Salmonella* was detected in 2033 meat samples from the total randomized 4360 samples (46.62%). Meanwhile, 50.85% of 1642 samples (1642) were found with *Salmonella* in 2008 [2]. The numbers of contaminated samples were significantly different ($P < 0.05$) and tend to increase. Angkititrakul *et al.* [3] collected 158 samples of raw meat from 33 self-cooking Thai restaurants in Khon Kaen municipal area from July – October 2011. The collected samples were pork, beef and chicken readily prepared for self-grilling or self-boiling. They examined 3 important causative agents of food poisoning, *Salmonella enterica*, *Escherichia coli* and *Staphylococcus aureus* in all samples. The results revealed 44.00, 60.00 and 25.00% of the collected samples were contaminated with *S. enterica*, *E. coli* and *S. aureus* respectively. Meanwhile, 77% of all samples were found to have at least 1 of these three bacteria and 8% of them were detected with all bacteria. The contamination rates of *Salmonella*, *S. aureus* and *E. coli* were various in different types of meat, in pork sample (59.00, 69.00 and 33.00 % respectively), in beef (52.00, 56.00 and 28.00% respectively) and in chicken (18.00, 56.00 and 12.00% respectively). Regarding the meat type, pork possessed the highest rate of contamination, 87.00% followed by beef (78.00%) and chicken (64.00%).

As above, there are a large number of reports on the presence of *Salmonella* in meat products only e.g. pork, beef and chicken but it is rare to be associated with *Salmonella* in digestive tract of cattle. Due to the study by Petcharat Sakdinunt and Supaphon Meeboon [4], *S. enteritidis* and *S. typhimurium* isolated from meat collected in the western region of Thailand performed multi-drug resistance with different rates of susceptibility and types of antibiotics. This indicates that the use of various antibiotics in livestock industry is improperly controlled which might affect and cause problems related to public health in case the infection of these bacteria is no longer cured by available antibiotics.

Therefore, this research was to study the prevalence and antimicrobial resistance of *Salmonella* isolated from digestive tract of the cattle to provide information for treatment and appropriate drug selection including monitoring the antibiotic resistance situation and the outbreak of *Salmonella*.

2. Materials and Methods

The cross-sectional study was used in this research. The fresh excrement was collected from the cecum of 40 fattening beef cattle at the slaughterhouse in winter, summer and rainy seasons. The total samples were from 120 cattle. The excrement was homogenized in the cecum prior to being transferred into a new plastic bag. The amount of the sample was at least 50 g. The air inside the bag was expelled before sealing completely. The samples bags were kept in 4 °C containing ice to retain the temperature and sent to the laboratory at Department of Animal

Science, Faculty of Agricultural Technology, Sakon Nakhon Rajabhat University to examine the presence of *Salmonell*a.

Salmonella culture and isolation were conducted according to ISO6579:2002 /AMD 1:2007 standard protocol [5]. Then, the positive samples with *Salmonella* were identified for serogroup and serotype at Regional Medical Science Center 7, Khon Kaen, Department of Medical Sciences, Ministry of Public Health.

The antimicrobial susceptibility was performed to assay the sensitivity to antibiotics using agar disk diffusion method and evaluated according to Clinical and Laboratory Standards Institute (CLSI) [6]. Eleven antibiotics were used due to their mechanism of action e.g. 10 µg ampicillin (Amp), 30 µg cefotaxime (Ctx), 30 µg chloramphenicol (C), 5 µg ciprofloxacin (Cip), 10 µg gentamicin (Gn), 30 µg kanamycin (K), 30 µg nalidixic acid (Na), 10 µg penicillin (P), 10 µg streptomycin (S), 30 µg tetracycline (Te) and 25 µg sulfamethoxazole/trimethoprim (Sxt). Three to five of *Salmonella* colonies on TSA medium were transferred into TSB medium and incubated at 35 °C for 2 – 6 hours. The density of the bacteria was adjusted to McFarland No. 0.5 or 1.5 x 10^8 CFU/ml using 0.85% normal saline solution (NSS). A sterile cotton swab was dipped in the adjusted medium and thoroughly streaked onto Mueller Hinton Agar (Difco, USA) then left for 3-5 minute to let the surface of the medium dry. The discs containing the antibiotics were firmly placed onto the agar medium and incubated at 35 °C for 18 – 24 hours. Diameters (mm.) of inhibition zones were measured and compared with the breakpoints referred to CLSI [6] to evaluate whether the bacterial isolates were resistant (R) intermediate (I) and susceptible (S). The data were collected and recorded then descriptive statistics was employed to report the prevalence and antibiotic resistance of *Salmonella*.

3. Results and Discussion

The prevalence of Salmonella was 2.50% during rainy season i.e. 1 out of 40 collected samples was detected with *Salmonella* but not in summer and winter, 0.83% prevalence rate in all seasons (1/120) as shown in Table 1. In this study variations in the seasonal incidence of *Salmonella* may be related to animal husbandry practice such as animal feed and water. In Thailand, there has not been any report on the *Salmonella* prevalence inside the cecal part of the cattle. The finding of this research suggested the rate of prevalence during rainy season similar to the report by Somsanith [7]. They investigated the *Salmonella* prevalence in the cecum of the cattle in Lao PDR and found that the rate was 2.29 (3/131). Moreover, Abouzeed *et al*. [8] reported the abundance of this bacterium in cecum of cattle without fasting before slaughtering in Canada, 0.94% (1/106).

The identification of Salmonella serotype

According to the result, there was one serotype identified in the obtained *Salmonella* isolate which was *S.* Brunei. This differed from the report by Abouzeed *et al.* [8]. They found that the most abundant rate of *Salmonella* derived from ceca of cattle in the slaughterhouses in Canada was *S.* Typhimurium (63.60%) followed by *S*. Agona (18.20%).

Table 1 *Salmonella* isolated from the collected ceca of fattening beef cattle at the slaughterhouse of Phon Yang Kham Livestock Breeding Cooperatives during November 2014 – July 2015

Season	Number of collected samples	Number of contaminated sample (%)	Group	Serotype
Winter	40	0	-	-
Summer	40	0	-	-
Rainy	40	1 (2.50%)	C	Brunei
Total	120	1 (0.83%)		

Table 2 The antimicrobial susceptibility of *Salmonella* isolated from the collected ceca of fattening beef cattle at the slaughterhouse in Sakon Nakhon province during November 2014 – July 2015

Bacterial type	Diameter of inhibitory zone (mm)										
	Amp	Ctx	C	Cip	Gn	K	Na	P	S	T	Sxt
Salmanella Brunei	0	25	22	34	20	20	20	0	15	19	20
Interpretation	R	S	S	S	S	S	S	R	S	S	S

Amp: ampicillin (10 µg), Ctx: cefotaxime (30 µg), C: chloramphenicol (30 µg), Cip: ciprofloxacin (5 µg), Gn: gentamicin (10 µg), K: kanamycin (30 µg), Na: nalidixic acid (30 µg), P: penicillin (10 µg), S: streptomycin (10 µg),T: tetracycline (30 µg), Sxt: sulfamethoxazole/trimethoprim (25 µg)

Antimicrobial resistance of Salmonella

As the result of antimicrobial susceptibility test via disk diffusion technique, the *Salmonella* isolate resisted to ampicillin and penicillin as shown in Table 2. Ray et al. [9 – 10] report that *Samonella* spp. derived from dairy cattle were resistant to streptomycin (66.67%), tetracycline (66.67%), ampicillin (33.33%). Moreover, *Salmonella* Brunei from inpatient children in Hussein Dey hospital, Algeria was able to tolerate kanamycin, amikacin but susceptible to ciprofloxacin, tetracycline and chloramphenicol [11]. According to author's experience in public service and association with fattening beef cattle farmers, penicillin/streptomycin and oxytetracycline have often been suggested by livestock supportive and treatment officers of the Cooperative to cure illnesses in the cattle. This could therefore be the cause of drug tolerance in bacteria living inside the cattle digestive tract.

Meanwhile, the isolated *Salmonella* was susceptible to cefotaxime, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, tetracycline and sulfamethoxazole/trimethroprim. Thus, these antibiotics should be used to treat *Salmonella*-infected cattle (Table 2).

4. Conclusion

The prevalence of *Salmonella* isolated from cecum of fattening beef cattle at the slaughterhouse of Phon Yang Kham Livestock Breeding Cooperatives in Sakon Nakhon Province during November, 2014 – July, 2015 was 0.83% i.e. only 1 out of 120 samples were detected with *Salmonella*.

4.1 There was one serovar identified which was *S.* Brunei, group C according to the result from Regional Medical Science Center 7, Khon Kaen, Department of Medical Sciences, Ministry of Public Health.

4.2 The *Salmonella* isolate obtained from the only one sample resisted to ampicillin and penicillin but was susceptible to cefotaxime, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, tetracycline and sulfamethoxazole/trimethroprim.

5. Suggestions

As the results of susceptibility test, one serotype of *Salmonella* resisted to ampicillin and penicillin. Hence these antibiotics should be avoided to use in fattening beef cattle. Sulfamethoxazole/trimethoprim and gentamicin are then alternatives for treating illnesses in the cattle.

6. Acknowledgement

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